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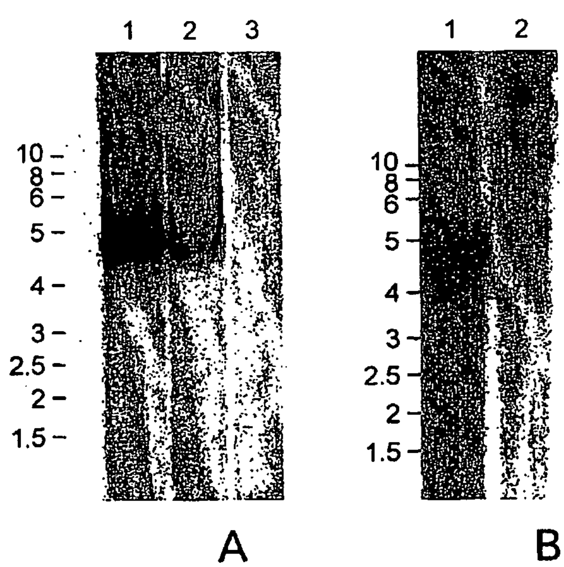
(54) **Title:** AVIAN ADENOASSOCIATED VIRUS (AAAV) AND USES THEREOF



(57) **Abstract:** The present invention provides an Avian adeno-associated virus (AAAV) virus and vectors and particles derived therefrom. In addition, the present invention provides methods of delivering a nucleic acid to a cell using the AAAV vectors and particles. Methods of isolating the AAAV are provided.

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AVIAN ADENOASSOCIATED VIRUS (AAAV) AND USES THEREOF

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention provides avian adeno-associated virus (AAAV) and vectors derived therefrom. Thus, the present invention relates to AAAV vectors for and methods of delivering nucleic acids to cells of subjects.

Background Art

To date, eight AAV isolates (AAV_{1-8}) have been, characterized and sequenced (2, 4, 19, 20, 25, 32, 51, 56) with AAV2 being the most extensively studied. AAV virions are approximately 20-25 nm in diameter and are composed of a mixture of assembled proteins (VPs) that encapsidate a linear ~4.7 kb single stranded DNA of plus or minus polarity (7, 43). The genome of AAVs is flanked by inverted terminal repeats (ITRs), which in the case of AAV2 are 145 nucleotides. The ITR is organized as three interrupted palindromes that can fold in an energetically favored T-shaped hairpin structure, which can exist in two orientations, termed flip and flop (42). The ITRs serve as origin of replication and contain *cis* acting elements required for rescue, integration, excision from cloning vectors and packaging (41, 42, 49 and 58).

The genetic map of the AAVs has been derived primarily from studies of AAV2 but is conserved in all serotypes (26, 27, 29, 36, 42, 45, 46, 58, 60, and 64). Two major open reading frames (*rep* and *cap* ORFs) and three transcriptional active promoters (P_5, P_{19}, P_{40}) have been identified in the genome of AAV2. The P_5 and P_{19} promoters encode for the nonstructural replication proteins Rep78 and Rep 68 and Rep 52 and Rep 40, respectively. Due to differential splicing, Rep78 and Rep52 have different C termini from Rep68 and Rep40. Transcription initiation from two promoters results in

Rep78 and Rep68 having different N termini from Rep52 and Rep 40. The P_{40} promoter transcribes two alternatively spliced mRNAs. The major mRNA species encodes for the major capsid protein VP3 from a conventional AUG codon and the minor capsid protein VP2 from an upstream in frame ACG codon. The minor mRNA species encodes the entire *cap* ORF to produce the minor capsid protein VP1 (47). VP1, VP2 and VP3 are found in a ratio of 1:1:10, respectively, and this stoichiometry is generated by the high abundance of one of the mRNA species and the low translation efficiency from an ACG codon in the case of VP2 (14, 47, 55). Previous studies have indicated that VP2 and VP3 are sufficient for particle formation and accumulation of encapsidated ssDNA progeny, while VP1 is required for assembly of highly infectious particles (63, 64).

All four Rep proteins possess NTP binding activity, DNA helicase activity and nuclear localization sequences, however only Rep78/68 possess DNA binding ability (33, 34, 66). Mutant AAV defective for the synthesis of the small Rep proteins (Rep52/40) are able to replicate DNA but no ssDNA progeny is encapsidated (16). The ability of Rep78/68 to bind and nick DNA in a sequence and strand specific manner inside the ITR is essential in every phase of the AAV life cycle, namely DNA replication, AAV gene expression, rescue from the integrated state and self-excision from cloning vectors (29, 35, 44). Nicking of the DNA within the ITR at the terminal resolution site (*trs*) requires binding of Rep78/68 proteins to a motif composed of tandem repeats of GAGY.

Among AAV serotypes, AAV1, 4, 7 and 8 are believed to be of simian origin while AAV2, 3 and 5 are from humans. AAV6 was found in a human adenovirus preparation and is very similar to AAV1. AAVs have also been reported in other mammalian species including canines, bovine, ovine and equine (8). An avian AAV was first isolated from the Olson strain of quail bronchitis adenovirus (68). It was later found that 50% of adenoviral field isolates from chickens in US and Ireland contained AAAVs serologically indistinguishable from the initial isolate (24). The AAAV was

found to be 20 nm in diameter, serologically distinct from AAV_{1-4}, did not agglutinate erythrocytes from several species tested and required adenovirus or herpes virus for replication (5, 68). In addition, AAAV was found to inhibit replication of several avian adenovirus and herpes virus (5, 52, 53). Physicochemical studies revealed that the capsid of AAAV consists of three VP proteins similar to other AAVs. The buoyant density of AAAV in CsCl gradients (1.39-1.44 g/cm^3) is similar to what have been reported for all AAVs (6, 30, 68).

The ability of AAV vectors to infect dividing and non-dividing cells and establish long-term transgene expression and the lack of pathogenicity has made them attractive for use in gene therapy applications. Recent evidence has indicated lack of cross competition in binding experiments suggesting that each AAV serotype may have a distinct mechanism of cell entry. Comparison of the *cap* ORFs from different serotypes has identified blocks of conserved and divergent sequence, with most of the later residing on the exterior of the virion, thus explaining the altered tissue tropism among serotypes (19-21, 48, 56). Vectors based on new AAV serotypes may have different host range and different immunological properties, thus allowing for most efficient transduction in certain cell types. In addition, characterization of new serotypes will aid in identifying viral elements required for altered tissue tropism.

Serological studies have provided evidence of avian adeno-associated virus infection in humans (69). Six percent of an unselected adult population was found positive for antibody to AAAV by agar gel precipitation (AGP), and 15.6% was positive by virus neutralization (VN). Fourteen percent of poultry workers (industry or research) were positive for AAAV antibody by AGP and 66% were positive by VN. In the same studies, no cross reaction was noted by AGP when antiserum to AAAV was reacted against primate antigens of serotypes 1-4 or when antiserum to AAV serotypes 1-4 were reacted against AAAV antigen. In addition, antiserum prepared against primate AAV1-4 did not neutralize the avian AAV. These results show that AAAV is a distinct serotype and infections are not restricted to avian species but are found in the

human adult population.

Based on the genome organization and sequence homology among insect densovirus, rodent parvovirus and human dependovirus, it has been previously proposed these virus may have diverged from a common ancestor and evolved strictly in their hosts (3). However, the high sequence homology between avian autonomous parvovirus and primate AAVs and the epidemiological documentation of AAAV transmission to humans provide evidence for host-independent evolution of at least some parvovirus genera. To better understand the relationship between the avian and the primate AAVs, the complete viral genome of AAAV was cloned and sequenced and used to generate recombinant viral particles.

The present invention provides the first complete genomic AAAV sequence. The genome of AAAV is 4,694 nucleotides in length and has similar organization with that of other AAVs. The entire genome of AAAV displays 56-65% identity at the nucleotide level with the other known AAVs. The AAAV genome has inverted terminal repeats of 142 nucleotides with the first 122 forming the characteristic T-shaped palindromic structure. The putative Rep-binding element (RBE) consists of a tandem $(GAGY)_4$ repeat, and the putative terminal resolution site (*trs*), CCGGT/CG, contains a single nucleotide substitution relative to the AAV_2 *trs*. Surprisingly and in contrast to AAV5, the AAAV ITR can be used as an origin or replication by either AAV5 or AAV2 Rep proteins for packaging. Thus the AAAV ITR can act as a universal ITR. The Rep ORF of AAAV displays 50-54 % identity at the amino acid level with the other AAVs, with most of the diversity clustered at the carboxyl and amino termini. Comparison of the capsid proteins of AAAV and the primate dependoviruses indicate divergent regions are localized to surface exposed loops. Despite these sequence differences, recombinant AAAV particles were produced carrying a *lacZ* reporter gene by co-transfection in 293T cells and transduction efficiency was examined in both chicken primary cells and several cell lines. This unique tropism allows AAAV to be useful as a vector for the development of transgenic

animals and also allows for the vaccination of eggs as well as the preparation of recombinant proteins in avian cultures. The exposed regions of AAAV are also sites for insertions of epitopes for the purpose of changing the tropism of the virus or antigen presentation. The present invention shows that AAAV is the most divergent adeno-associated virus described to date, but maintains all the characteristics unique to the genera of dependovirus.

The present invention provides a vector comprising the AAAV virus or a vector comprising subparts of the virus, as well as AAAV viral particles. While AAAV is similar to primate AAVs, the viruses are found herein to be physically and genetically distinct. These differences endow AAAV with some unique properties and advantages which better suit it as a vector for gene therapy or gene transfer applications. As shown herein, AAAV capsid protein, again surprisingly, is distinct from primate capsid protein and exhibits different tissue tropism, thus making AAAV capsid-containing particles suitable for transducing cell types for which primate AAVs are unsuited or less well-suited. AAAV is serologically distinct and thus, in a gene therapy application, AAAV would allow for transduction of a patient who already possesses neutralizing antibodies to primate isolates either as a result of natural immunological defense or from prior exposure to other vectors. AAAV is also useful for gene transfer to other species for the development of transgenic animals or the production of vaccines and recombinant proteins in eggs. Thus, the present invention, by providing these new recombinant vectors and particles based on AAAV, provides a new and highly useful series of vectors.

SUMMARY OF THE INVENTION

The present invention provides a nucleic acid vector comprising a pair of avian adeno-associated virus (AAAV) inverted terminal repeats and a promoter between the inverted terminal repeats.

The present invention further provides an AAAV particle containing a vector comprising a pair of AAAV inverted terminal repeats.

The present invention further provides an AAAV particle containing a vector comprising a pair of AAV2 inverted terminal repeats.

The present invention further provides an AAAV particle containing a vector comprising a pair of AAV5 inverted terminal repeats.

The present invention further provides an AAV1 particle containing a vector comprising a pair of AAAV inverted terminal repeats.

The present invention further provides an AAV2 particle containing a vector comprising a pair of AAAV inverted terminal repeats.

The present invention further provides an AAV3 particle containing a vector comprising a pair of AAAV inverted terminal repeats.

The present invention further provides an AAV4 particle containing a vector comprising a pair of AAAV inverted terminal repeats.

The present invention further provides an AAV5 particle containing a vector comprising a pair of AAAV inverted terminal repeats.

The present invention further provides an AAV6 particle containing a vector comprising a pair of AAAV inverted terminal repeats

The present invention further provides an AAV7 particle containing a vector comprising a pair of AAAV inverted terminal repeats

The present invention further provides an AAV8 particle containing a vector comprising a pair of AAAV inverted terminal repeats

The present invention further provides a dependovirus particle containing a vector comprising a pair of AAAV inverted terminal repeats.

Additionally, the instant invention provides an isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:1 (AAAV genome). Furthermore, the present invention provides an isolated nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO:1 (AAAV genome).

The present invention provides an isolated nucleic acid encoding an AAAV Rep protein, for example, the nucleic acid as set forth in SEQ ID NO:2. Additionally provided is an isolated full-length AAAV Rep protein or a unique fragment thereof. Additionally provided is an isolated AAAV Rep 42 protein having the amino acid sequence set forth in SEQ ID NO:9, or a unique fragment thereof. Additionally provided is an isolated AAAV Rep 52 protein having the amino acid sequence set forth in SEQ ID NO:5, or a unique fragment thereof. Additionally provided is an isolated AAAV Rep 68 protein, having the amino acid sequence set forth in SEQ ID NO:7 or a unique fragment thereof. Additionally provided is an isolated AAAV Rep 78 protein having the amino acid sequence set forth in SEQ ID NO:3, or a unique fragment thereof. The sequences for these proteins are provided below in the Sequence Listing and elsewhere in the application where the proteins are described.

The present invention further provides an isolated AAAV capsid protein, VP1, having the amino acid sequence set forth in SEQ ID NO:11, or a unique fragment thereof. Additionally provided is an isolated AAAV capsid protein, VP2, having the amino acid sequence set forth in SEQ ID NO:13, or a unique fragment thereof. Also provided is an isolated AAAV capsid protein, VP3, having the amino acid sequence set forth in SEQ ID NO:15, or a unique fragment thereof.

The present invention additionally provides an isolated nucleic acid encoding AAAV capsid protein, for example, the nucleic acid set forth in SEQ ID NO:10, or a unique fragment thereof.

The present invention further provides an AAAV particle comprising a capsid protein consisting essentially of the amino acid sequence set forth in SEQ ID NO:11, or a unique fragment thereof.

Additionally, provided by the present invention is an isolated nucleic acid comprising an AAAV p5 promoter having the nucleic acid sequence set forth in SEQ ID NO:22, or a unique fragment thereof.

The instant invention provides a method of screening a cell for infectivity by AAAV comprising contacting the cell with AAAV and detecting the presence of AAAV in the cells.

The present invention further provides a method of delivering a nucleic acid to a cell comprising administering to the cell an AAAV particle containing a vector comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, thereby delivering the nucleic acid to the cell.

The present invention also provides a method of delivering a nucleic acid to a subject comprising administering to a cell from the subject an AAAV particle comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, and returning the cell to the subject, thereby delivering the nucleic acid to the subject.

The present invention also provides a method of delivering a nucleic acid to a cell in a subject comprising administering to the subject an AAAV particle comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, thereby

delivering the nucleic acid to a cell in the subject.

The instant invention further provides a method of delivering a nucleic acid to a cell in a subject having antibodies to other serotypes of AAV comprising administering to the subject an AAAV particle comprising the nucleic acid, thereby delivering the nucleic acid to a cell in the subject.

The present invention also provides a method of delivering a nucleic acid to a subject comprising administering to a cell from the subject an AAAV particle comprising the nucleic acid inserted between a pair of AAAV inverted terminal repeats, and returning the cell to the subject, thereby delivering the nucleic acid to the subject.

The present invention also provides a method of delivering a nucleic acid to a cell in a subject comprising administering to the subject an AAAV particle comprising the nucleic acid inserted between a pair of AAAV inverted terminal repeats, thereby delivering the nucleic acid to a cell in the subject.

The instant invention further provides a method of delivering a nucleic acid to a cell in a subject having antibodies to primate AAVs comprising administering to the subject an AAAV particle comprising the nucleic acid, thereby delivering the nucleic acid to a cell in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a Southern blot analysis of AAAV nuclease resistant particles in 293T and LMH cells. A) 293T cells were transfected with pAAAV alone (lane 3), pAAAV plus pAd12 (lane 2) and pAAAV plus infection with wt Ad (lane 1). B) LMH cells were transfected with pAAAV alone (lane 2) or pAAAV plus infection with FAV1 (lane 1). Viral DNA was isolated as described in Materials and Methods and fractionated on agarose gel before southern blot analysis with a 32P-labeled pAAAV

DNA.

Figure 2 shows the AAAV ITR. The sequence of the ITR is shown in the hairpin conformation. The putative Rep binding site is boxed, while the putative *trs* is underlined and the cleavage site is indicated by an arrow.

Figure 3 is the sequence of an AAAV genome. The genomes of AAAV, AAV2, AAV4 and AAV5 were aligned using Clustal W. The sequences of the ITRs are presented in italics. The putative *trs* is indicated by vertical arrow and the putative RBS is underlined. Proposed transcription factor binding sites and the polyadenylation signal are also underlined. Proposed transcription initiation sites of the p5, p19 and p40 promoters and splice donor and acceptor sites are indicated by horizontal arrows. Initiation and termination codons are presented in bold letters.

Figures 4A and 4B illustrate comparisons of *rep* and *cap* ORFs. The *rep* and *cap* ORFs of AAAV, AAV2, AAV4, AAV5 and Goose autonomous parvovirus (GP) were aligned using Clustal W. Identical amino acids are indicated by a dot. Dashes indicate gaps in the sequence added by the alignment program. A) Horizontal arrows indicate the initiator codon of the p5 and p19 Rep proteins. The Rep endonuclease site established by Tyr155 and the tetrahedrally coordinated Asp24, Glu83, His90 and His92 are presented in bold letters and are over lined by an asterisk. The region important for Rep multimerization, the ATP binding site and the basic amino acids of the nuclear localization signal are underlined. The zinc finger motifs in the carboxy terminus are underlined and the coordinating cystine and histidine residues are indicated by dots. B) The theoretical initiator codons of VP2 and VP3 are indicated in bold letters. Regions that have been proposed to be on the surface of AAV2 are underlined and divergent regions are boxed. The heparin binding region in the capsid of AAV2 is also indicated.

Figures 5A and 5B show vector constructs for generation of recombinant AAAV virus and transduction of chicken fibroblasts. A) Wild type AAAV, vector

plasmid (pA3Vbgal) and production yields of rAAAV using helper plasmids providing the rep gene under control of CMV, MMTV or the native P5 promoter. The helper plasmids pCA3VRC, pMA3VRC, pA3VRC were individually co-transfected with pA3Vbgal and an adenovirus helper plasmid in 293T cells and rAAAV was produced as described in Material and Methods. The number of rAAAV genomes produced in each group was determined by quantitative PCR and is expressed as DNAse resistant particle/cell (DPN/cell). ITR: inverted terminal repeats from AAAV, RSV: Rous Sarcoma virus long terminal repeat promoter, CMV: cytomegalovirus immediate early promoter, MMTV murine leukemia virus long terminal repeat promoter, β-Gal: β–galactosidase gene, SV40-polyA: polyadenylation signal from SV40. B) Relative transduction efficiency of primary chicken embryonic fibroblasts (CEF) and immortalized chicken embryonic fibroblasts (DF1) with equal particles of rAAAV expressing LacZ.

Figure 6 shows results with neuraminidase indicating that while AAV5 is sensitive to sialic acid treatment AAAV is not.

Figure 7 shows that while AAV2 is sensitive to heparin competition, AAAV is not.

Figure 8 shows the role of terminal lactose in AAAV binding by incubating virus with different conjugates that had either terminal lactose or sialic acid. AAV5 is sensitive to competition with sialic acid conjugates but AAAV is not. However AAAV is competed by terminal lactose conjugates confirming ERCR lectin result.

Figures 9A and 9B show that treatment with tunicamycin blocks virus binding and transduction, suggesting that glycosylation is N-linked. AAV5 is the control.

Figure 10 confirms that glycoprotein is involved in AAAV binding and transduction, cells were treated with broad specificity protease, trypsin. Like AAV5,

trypsin minimally effects virus binding. However treatment with low levels of tunicamycin dramatically increased the inhibition in binding observed with trypsin treatment..

Figures 11A and 11B show that the linkage is probably not an O-linkage.

Figures 12A and 12B show results with a series of N-linked inhibitors: NB-DNJ is a specific inhibitor of ER glucosidase I, II, and glycolipid; NB-DGJ glycolipid synthesis inhibiting properties as NB-DNJ; DNJ inhibits glucosidase 1,2; Fumonisin B1 is an inhibitor of ceramide synthesis; and PDMP is an inhibitor of glycosphingolipid synthesis.

Figures 13A and 13B show results using several lectins and confirms previous results that sialic acid is not important (WGA vs WGA-s). *Erythrina corralodendron* (ERCL) which binds terminal poly lactose does inhibit suggesting that the virus is binding terminal lactose.

DETAILED DESCRIPTION OF THE INVENTION

As used in the specification and in the claims, "a" can mean one or more, depending upon the context in which it is used. The terms “having” and “comprising” are used interchangeably herein, and signify open ended meaning.

The present application provides a recombinant avian adeno-associated virus (AAAV). This virus has one or more of the characteristics described below. The compositions of the present invention do not include wild-type AAAV. The methods of the present invention can use either wild-type AAAV or recombinant AAAV-based delivery.

The present invention provides novel AAAV particles, recombinant AAAV vectors, recombinant AAAV virions and novel AAAV nucleic acids and polypeptides. An AAAV particle is a viral particle comprising an AAAV capsid protein. A recombinant AAAV vector is a nucleic acid construct that comprises at least one unique nucleic acid of AAAV. A recombinant AAAV virion is a particle containing a recombinant AAAV vector, wherein the particle can be either an AAAV particle as described herein or a non-AAAV particle. Alternatively, the recombinant AAAV virion is an AAAV particle containing a recombinant vector, wherein the vector can be either an AAAV vector as described herein or a non-AAAV vector. These vectors, particles, virions, nucleic acids and polypeptides are described below.

The present invention provides the nucleotide sequence of the AAAV genome and vectors and particles derived therefrom. Specifically, the present invention provides a nucleic acid vector comprising a pair of AAAV inverted terminal repeats (ITRs) and a promoter between the inverted terminal repeats. The rep proteins of AAV2 and AAV5 or AAAV will bind to the AAAV ITR and the AAAV IRF can function as a universal origin or replication for packaging of recombinant AAV particles. The minimum sequence necessary for this activity is the TRS site (SEQ ID NO: 20) where Rep cleaves in order to replicate the virus. Minor modifications in an ITR are contemplated and are those that will not interfere with the hairpin structure formed by the ITR as described herein and known in the art. Furthermore, to be considered within the term e.g. it must retain the Rep binding site described herein. One of skill in the art would know how to modify an AAAV ITR such that the hairpin structure is maintained and the Rep binding site is present. One of skill in the art could contemplate any ITR that contains a Rep binding site (SEQ ID NO: 21) and a trs site (SEQ ID NO: 20). Such an ITR could be utilized in any of the vectors described herein.

The D region of the AAAV ITR, a single stranded region of the ITR, inboard of the TRS site, has been shown to bind a factor which depending on its phosphorylation

state correlates with the conversion of the AAV from a single stranded genome to a transcriptionally active form that allows for expression of the viral DNA. This region is conserved between AAV2, 3, 4,and 6 but is divergent in AAV5 and AAAV. The D+ region (SEQ ID NO: 18) is the reverse complement of the D- region (SEQ ID NO: 19).

The promoter can be any desired promoter, selected by known considerations, such as the level of expression of a nucleic acid functionally linked to the promoter and the cell type in which the vector is to be used. That is, the promoter can be tissue/cell-specific. Promoters can be prokaryotic, eukaryotic, fungal, nuclear, mitochondrial, viral or plant promoters. Promoters can be exogenous or endogenous to the cell type being transduced by the vector. Promoters can include, for example, bacterial promoters, known strong promoters such as SV40 or the inducible metallothionein promoter, or an AAV promoter, such as an AAV p5 promoter. Additionally, chimeric regulatory promoters for targeted gene expression can be utilized. Examples of these regulatory systems, which are known in the art, include the tetracycline based regulatory system which utilizes the tet transactivator protein (tTA), a chimeric protein containing the VP16 activation domain fused to the tet repressor of *Escherichia coli*, the IPTG based regulatory system, the CID based regulatory system, and the Ecdysone based regulatory system (44). Other promoters include promoters derived from actin genes, immunoglobulin genes, cytomegalovirus (CMV), adenovirus, bovine papilloma virus, adenoviral promoters, such as the adenoviral major late promoter, an inducible heat shock promoter, respiratory syncytial virus, Rous sarcomas virus (RSV), etc.

Specifically, the promoter can be an AAV2 p5 promoter or an AAV5 p5 promoter or an AAAV p5 promoter. More specifically, the AAAV p5 promoter can be at about the same location in SEQ ID NO: 1 as the AAV2 p5 promoter, in the corresponding AAV2 published sequence. Additionally, the p5 promoter may be enhanced by nucleotides 1-142 of SEQ ID NO:1. Furthermore, smaller fragments of the p5 promoter that retain promoter activity can readily be determined by standard procedures including, for example, constructing a series of deletions in the p5

promoter, linking the deletion to a reporter gene, and determining whether the reporter gene is expressed, *i.e.*, transcribed and/or translated. The promoter can be the promoter of any of the AAV serotypes, and can be the p19 promoter (SEQ ID NO: 23) or the p40 promoter set forth in the sequence listing as SEQ ID NO: 24.

It should be recognized that any errors in any of the nucleotide sequences disclosed herein can be corrected, for example, by using the hybridization procedure described below with various probes derived from the described sequences such that the coding sequence can be reisolated and resequenced. Rapid screening for point mutations can also be achieved with the use of polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) (43). The corresponding amino acid sequence can then be corrected accordingly.

The AAAV-derived vector of the invention can further comprise a heterologous nucleic acid functionally linked to the promoter. By "heterologous nucleic acid" is meant that any heterologous or exogenous nucleic acid, i.e. not normally found in wild-type AAAV can be inserted into the vector for transfer into a cell, tissue or organism. By "functionally linked" is meant that the promoter can promote expression of the heterologous nucleic acid, as is known in the art, and can include the appropriate orientation of the promoter relative to the heterologous nucleic acid. Furthermore, the heterologous nucleic acid preferably has all appropriate sequences for expression of the nucleic acid. The nucleic acid can include, for example, expression control sequences, such as an enhancer, and necessary information processing sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences.

The heterologous nucleic acid can encode beneficial proteins or polypeptides that replace missing or defective proteins required by the cell or subject into which the vector is transferred or can encode a cytotoxic polypeptide that can be directed, *e.g.*, to cancer cells or other cells whose death would be beneficial to the subject. The

heterologous nucleic acid can also encode antisense RNAs that can bind to, and thereby inactivate, mRNAs made by the subject that encode harmful proteins. The heterologous nucleic acid can also encode ribozymes that can effect the sequence-specific inhibition of gene expression by the cleavage of mRNAs. In one embodiment, antisense polynucleotides can be produced from a heterologous expression cassette in an AAAV vector construct where the expression cassette contains a sequence that promotes cell-type specific expression (Wirak *et al.*, *EMBO* 10:289 (1991)). For general methods relating to antisense polynucleotides, see *Antisense RNA and DNA*, D. A. Melton, Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1988).

Examples of heterologous nucleic acids which can be administered to a cell or subject as part of the present AAAV vector can include, but are not limited to the following: nucleic acids encoding secretory and non-secretory proteins, nucleic acids encoding therapeutic agents, such as tumor necrosis factors (TNF), such as TNF-α; interferons, such as interferon-α, interferon-β, and interferon-γ; interleukins, such as IL-1, IL-1β, and ILs -2 through -14; GM-CSF; adenosine deaminase; cellular growth factors, such as lymphokines; soluble CD4; Factor VIII; Factor IX; T-cell receptors; LDL receptor; ApoE; ApoC; alpha-1 antitrypsin; ornithine transcarbamylase (OTC); cystic fibrosis transmembrane receptor (CFTR); insulin; Fc receptors for antigen binding domains of antibodies, such as immunoglobulins; anti-HIV decoy tar elements; and antisense sequences which inhibit viral replication, such as antisense sequences which inhibit replication of hepatitis B or hepatitis non-A, non-B virus. The nucleic acid is chosen considering several factors, including the cell to be transfected. Where the target cell is a blood cell, for example, particularly useful nucleic acids to use are those which allow the blood cells to exert a therapeutic effect, such as a gene encoding a clotting factor for use in treatment of hemophilia. Another target cell is the lung airway cell, which can be used to administer nucleic acids, such as those coding for the cystic fibrosis transmembrane receptor, which could provide a gene therapeutic treatment for cystic fibrosis. Other target cells include muscle cells where useful

nucleic acids, such as those encoding cytokines and growth factors, can be transduced and the protein the nucleic acid encodes can be expressed and secreted to exert its effects on other cells, tissues and organs, such as the liver. Furthermore, the nucleic acid can encode more than one gene product, limited only, if the nucleic acid is to be packaged in a capsid, by the size of nucleic acid that can be packaged.

Furthermore, suitable nucleic acids can include those that, when transferred into a primary cell, such as a blood cell, cause the transferred cell to target a site in the body where that cell's presence would be beneficial. For example, blood cells such as TIL cells can be modified, such as by transfer into the cell of a Fab portion of a monoclonal antibody, to recognize a selected antigen. Another example would be to introduce a nucleic acid that would target a therapeutic blood cell to tumor cells. Nucleic acids useful in treating cancer cells include those encoding chemotactic factors which cause an inflammatory response at a specific site, thereby having a therapeutic effect.

Cells, particularly blood cells, muscle cells, airway epithelial cells, brain cells and endothelial cells having such nucleic acids transferred into them can be useful in a variety of diseases, syndromes and conditions. For example, suitable nucleic acids include nucleic acids encoding soluble CD4, used in the treatment of AIDS and α-antitrypsin, used in the treatment of emphysema caused by α-antitrypsin deficiency. Other diseases, syndromes and conditions in which such cells can be useful include, for example, adenosine deaminase deficiency, sickle cell deficiency, brain disorders such as Alzheimer's disease, thalassemia, hemophilia, diabetes, phenylketonuria, growth disorders and heart diseases, such as those caused by alterations in cholesterol metabolism, and defects of the immune system.

As another example, hepatocytes can be transfected with the present vectors having useful nucleic acids to treat liver disease. For example, a nucleic acid encoding OTC can be used to transfect hepatocytes (*ex vivo* and returned to the liver or *in vivo*) to treat congenital hyperammonemia, caused by an inherited deficiency in OTC.

Another example is to use a nucleic acid encoding LDL to target hepatocytes *ex vivo* or *in vivo* to treat inherited LDL receptor deficiency. Such transfected hepatocytes can also be used to treat acquired infectious diseases, such as diseases resulting from a viral infection. For example, transduced hepatocyte precursors can be used to treat viral hepatitis, such as hepatitis B and non-A, non-B hepatitis, for example by transducing the hepatocyte precursor with a nucleic acid encoding an antisense RNA that inhibits viral replication. Another example includes transferring a vector of the present invention having a nucleic acid encoding a protein, such as α-interferon, which can confer resistance to the hepatitis virus.

For a procedure using transfected hepatocytes or hepatocyte precursors, hepatocyte precursors having a vector of the present invention transferred in can be grown in tissue culture, removed from the tissue culture vessel, and introduced to the body, such as by a surgical method. In this example, the tissue would be placed directly into the liver, or into the body cavity in proximity to the liver, as in a transplant or graft. Alternatively, the cells can simply be directly injected into the liver, into the portal circulatory system, or into the spleen, from which the cells can be transported to the liver via the circulatory system. Furthermore, the cells can be attached to a support, such as microcarrier beads, which can then be introduced, such as by injection, into the peritoneal cavity. Once the cells are in the liver, by whatever means, the cells can then express the nucleic acid and/or differentiate into mature hepatocytes which can express the nucleic acid.

The AAAV-derived vector can include any normally occurring AAAV sequences in addition to an ITR and promoter. Examples of vector constructs are provided below.

The present vector or AAAV particle or recombinant AAAV virion can utilize any unique fragment of these present AAAV nucleic acids, including the AAAV nucleic acids set forth in SEQ ID NOS: 1, 2, 4, 6, 8, 10, 12, 14, and 16-24. To be

unique, the fragment must be of sufficient size to distinguish it from other known sequences, most readily determined by comparing any nucleic acid fragment to the nucleotide sequences of nucleic acids in computer databases, such as GenBank. Such comparative searches are standard in the art. In particular, one of skill in the art will know how to distinguish an AAAV sequence from other AAV sequences. Therefore, the present invention provides AAAV nucleic acid sequences that are not found in other AAV sequences.

For example, one of skill in the art could perform alignments with an alignment program such as ClustalW or Blast2 where the parameters would be GAPOPEN or OPENGAP or OPEN GAP PENALTY : Penalty for the first residue in a gap (e.g., fasta defaults: -12 by with proteins, -16 for DNA). GAPEXT or EXTENDGAP or EXTEND GAP PENALTY : Penalty for additional residues in a gap (e.g. fasta defaults: -2 with proteins, -4 for DNA). Thus, if would be routine for one of skill in the art to utilize such alignment programs for identification of unique sequences as well as sequences that are 50%, 60%, 70%, 80%, 90%, 95% and 100% identical to the nucleic acid sequences described herein, as well as sequences that are 50%, 60%, 70%, 80%, 90%, 95% and 100% identical to the protein sequences described herein.

Typically, a unique fragment useful as a primer or probe will be at least about 8 or 10, preferable at least 20 or 25 nucleotides in length, depending upon the specific nucleotide content of the sequence. Additionally, fragments can be, for example, at least about 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675 or 700 nucleotides in length and can encode polypeptides or be probes. The nucleic acid can be single or double stranded, depending upon the purpose for which it is intended. Where desired, the nucleic acid can be RNA.

The present invention further provides an AAAV capsid protein to contain the vector. In particular, the present invention provides not only a polypeptide comprising

all three AAAV coat proteins, *i.e.*, VP1, VP2 and VP3, but also a polypeptide comprising each AAAV coat protein individually, SEQ ID NOS: 11, 13, and 15, respectively. Thus an AAAV particle comprising an AAAV capsid protein comprises at least one AAAV coat protein VP1, VP2 or VP3. The present invention also provides particles comprising fragments of VP1, VP2 or VP3 that allow the particle to maintain AAAV functionality and tropism. An AAAV particle comprising an AAAV capsid protein can be utilized to deliver a nucleic acid vector to a cell, tissue or subject. For example, the herein described AAAV vectors can be encapsidated in an AAV5 capsid-derived particle and utilized in a gene delivery method. Furthermore, other viral nucleic acids can be encapsidated in the AAAV particle and utilized in such delivery methods. For example, an AAV1-8 vector (e.g. a vector comprising an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 or an AAV8 ITR and a nucleic acid of interest) can be encapsidated in an AAAV particle and administered. Furthermore, an AAAV chimeric capsid incorporating both AAV2 capsid and AAAV capsid sequences can be generated, by standard cloning methods, selecting regions from the known sequences of each protein as desired. For example, particularly antigenic regions of the AAAV capsid protein can be replaced with the corresponding region of the AAV2 capsid protein. In addition to chimeric capsids incorporating AAV2 capsid sequences, chimeric capsids incorporating AAV1, AAV3-8, and/or AAV5 capsid sequences can be generated, by standard cloning methods, selecting regions from the known sequences of each protein as desired. Alternatively a chimeric capsid can be made by the addition of a plasmid that expresses AAV1-8 capsid proteins at a ratio with the AAAV capsid expression plasmid that allows only a few capsid proteins to be incorporated into the AAAV particle. Thus, for example, a chimeric particle may be constructed that contains 6 AAV2 capsid proteins and 54 AAAV capsid proteins if the complete capsid contains 60 capsid proteins.

The AAAV capsid proteins can also be modified to alter their specific tropism by genetically modifying the capsid to comprise a specific ligand that binds to a cell surface receptor. Alternatively, the capsid can be chemically modified by conjugating a

ligand to a cell surface receptor. By genetically or chemically altering the capsids, the tropism can be modified to direct AAAV to a particular cell or population of cells. The capsids can also be altered immunologically by conjugating the capsid to an antibody that recognizes a specific protein on the target cell or population of cells.

It has been recently reported that insertion of foreign epitopes (RGD motif, LH receptor targeting epitope) in certain regions of AAV2 capsid can redirect viral tropism. However, AAV2 naturally infects a wide variety of cell types and complete retargeting of rAAV2 would be difficult to achieve. For example, removal of the heparin binding activity, which is a major determinant of aav2 transduction in vitro, still results in AAV2 transduction of heart tissue in vivo. rAAAV displays a more restrict tropism with preferential transduction of avian cells. Therefore AAAV could be more easily engineered to specifically target certain cell types. Based on cryo-electron microscopy imaging of AAV2, 4 and 5, molecular modeling, and sequence alignments, we have identified regions in the capsid of AAAV that are on the virus surface and could tolerate substitution. Two of these regions are aa 269-278 (PSGGDNNNKF), and for some uses, more preferably aa 267-274 (QGPSGGD). There is evidence that the variable loop comprises QGPSGGD and that NNNKF may be conserved and may be important in structure. A substitution into PSGGDNNNKF it did not assemble well and was not infectious in any cell. However, this type of insertion is useful for antigen presentation but not retargeting of the vector. Antigens presented in order arrays on the surface of viruses tend to be more antigenic than if they are presented in random. Another region is aa 454-463 (VSQAGSSGRA).

For insertion between aa 146-147

AAAV 146-LH nc: CGTCTTTGAGTCTTCCACCAGACCAAAG

AAAV 146-LH c:
CACTGCAGCACCTGCTACTACCACAAGAGCGCTCCGACCGGAGACAAG
CG

For substitution at aa 267-274,

LHR-267-274F

5'CAACCACCTGTACAAACGAATCCACTGCAGCACCTGCTACTACCACAAGA GCAACAACAACAAATTCTTTGGATTC-3'

LHR-267-274R

5'GAATCCAAAGAATTTGTTGTTGTTGCTCTTGTGGTAGTAGCAGGTGCTGCA GTGGATTCGTTTGTACAGGTGGTTG-3'

For substitution at aa 269-278,

LHR-269-278F

CAAACGAATCCAAGGACACTGCAGCACCTGCTACTACCACAAGAGCTTTGG ATTCAGCACC

LHR-269-278R

GGTGCTGAATCCAAAGCTCTTGTGGTAGTAGCAGGTGCTGCAGTGTCCTTG GATTCGTTTG

For substitution at aa 454-463,

LHR-454-463F

TACCTCTGGGCTTTCAGCTCCCACTGCAGCACCTGCTACTACCACAAGAGCC TTCATTACTCGCGGGCGAC

LHR-454-463R

GTCGCCCGCGAGTAATGAAGGCTCTTGTGGTAGTAGCAGGTGCTGCAGTGG GAGCTGAAAGCCCAGAGGTA

Other regions of the AAAV capsid could also accommodate the substitution of amino acids that would allow for epitope presentation on the surface of the virus. All of these regions would have the following characteristics in common: 1) surface exposure, 2) ability to support a substitution of sequence to insert the epitope, 3) allows for capsid assembly. Examples of other insertion or substitution regions on the virus surface are the regions around T385-R394, S588-R601, T589-R600, S455-R462, S455-R463, T546-Q559, R550-T556, V329-I338, G708-T720, Ser 710-Y728 of VP1.

Because of the symmetry of the AAV particles, a substitution in one subunit of the capsid will appear multiple times on the capsid surface. For example the capsid is made of approximately 55 VP3 proteins (i.e., 50 VP3 is 90% of the capsid and there are 60 faces on an icosahedron). Therefore an epitope incorporated in the VP3 protein could be expressed 55 times on the surface of each particle increasing the likelihood of the epitope forming a stable interaction with its target. An epitope inserted upstream of the VP3 ORF may be presented in both the VP2 and VP1 proteins, or up to 10 times on the surface of each particle. In some cases this ligand density may be too high for functional binding or this high a density of epitope may interfere with capsid formation. The epitope density could be lowered by introducing another plasmid into the packaging system for production of recombinant particles and the ratio between the packaging plasmid with the modified VP protein and the wild type VP protein altered to balance the epitope density on the virus surface. Thus one example would be on an epitope that is targeted for the mounds at the 3 fold axis of symmetry. By mixing in 2 wild type rep and cap expression plasmids with 1 mutant rep and cap plasmid, instead of the mutant epitope being found in all three mounds at each three fold axis (60 times), it will only be present in 1 mound (20 times).

Epitopes can be incorporated into the virus capsid for the purpose of 1) altering the tropism of the virus, 2) blocking an immune response directed at the virus, 3) developing a host immune response to the epitope for the purpose of vaccination, and 4) catalyzing a reaction.

Examples of epitopes that can be added to AAAV capsids include but are not limited to the following proteins and protein fragments:

LH receptor binding epitope: Photoaffinity labeling of the lutropin receptor with synthetic peptide for carboxyl terminus of the human choriogonadotropin alpha subunit. Kundu GC, Ji I, McCormick DJ, Ji TH. J Biol Chem. 1996 May

10;271(19):11063-6 (incorporated herein by reference);

RGD integrin binding epitope: Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. Pierschbacher MD, Ruoslahti E. Nature. 1984 May 3-9;309(5963):30-3 (incorporated herein by reference);

CD13 binding epitope NGRAHA: Incorporation of tumor-targeting peptides into recombinant adeno-associated virus capsids. Grifman M, Trepel M, Speece P, Gilbert LB, Arap W, Pasqualini R, Weitzman MD. Mol Ther. 2001 Jun;3(6):964-75 (incorporated herein by reference) and F. Curnis, A. Sacchi, L. Borgna, F. Magni, A. Gasparri and A. Corti, Enhancement of tumor necrosis factor alpha antitumor immunotherapeutic properties by targeted delivery to aminopeptidase N (CD13). Nat. Biotechnol. 18 (2000), pp. 1185*1190 (incorporated herein by reference);

Single chain antibody fragments: Q. Yang, Development of novel cell surface CD34-targeted recombinant adenoassociated virus vectors for gene therapy. Hum. Gene Ther. 9 (1998), pp. 1929*1937 (incorporated herein by reference);

Endothelial cell binding epitope SIGYPLP: R. Pasqualini and E. Ruoslahti, Organ targeting in vivo using phage display peptide libraries. Nature 380 (1996), pp. 364*366 (incorporated herein by reference) and D. Rajotte, W. Arap, M. Hagedorn, E. Koivunen, R. Pasqualini and E. Ruoslahti, Molecular heterogeneity of the vascular endothelium revealed by in vivo phage display. J. Clin. Invest. 102 (1998), pp. 430*437 (incorporated herein by reference);

Lung targeting peptide CGFECVRQCPERC: D. Rajotte and E. Ruoslahti, Membrane dipeptidase is the receptor for a lung-targeting peptide identified by in vivo phage display. J. Biol. Chem. 274 (1999), pp. 11593*11598 (incorporated herein by reference);

Muscle targeting peptide ASSLNIA: T. I. Samoylova and B. F. Smith, Elucidation of muscle-binding peptides by phage display screening. Muscle Nerve 22 (1999), pp. 460*466 (incorporated herein by reference);

Tumor endothelium targeting: W. Arap, R. Pasqualini and E. Ruoslahti, Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. Science 279 (1998), pp. 377*380 (incorporated herein by reference);

Major immunogenic epitope for parvovirus B19 NISLDNPLENPSSLFDLVARIK: K. Yoshimoto, A second neutralizing epitope of B19 parvovirus implicates the spike region in the immune response. J. Virol. 65 (1991), pp. 7056*7060 (incorporated herein by reference);

Serpin receptor ligand (KFNKPFVFLI): A small, synthetic peptide for gene delivery via the serpin-enzyme complex receptor. Patel S, Zhang X, Collins L, Fabre JW. J Gene Med. 2001 May-Jun;3(3):271-9 (incorporated herein by reference);

Hemagglutinin (HA) 91-108: A retro-inverso peptide analogue of influenza virus hemagglutinin B-cell epitope 91-108 induces a strong mucosal and systemic immune response and confers protection in mice after intranasal immunization. Ben-Yedidia T, Beignon AS, Partidos CD, Muller S, Arnon R. Mol Immunol. 2002 Oct;39(5-6):323-31 (incorporated herein by reference);

NDV epitope 447 to 455: Newcastle disease virus (NDV) marker vaccine: an immunodominant epitope on the nucleoprotein gene of NDV can be deleted or replaced by a foreign epitope. Mebatsion T, Koolen MJ, de Vaan LT, de Haas N, Braber M, Romer-Oberdorfer A, van den Elzen P, van der Marel P. J Virol. 2002 Oct;76(20):10138-46 (incorporated herein by reference);

RETANEF HIV-1 epitope vaccine candidate: A novel chimeric Rev, Tat, and Nef

(Retanef) antigen as a component of an SIV/HIV vaccine. Hel Z, Johnson JM, Tryniszewska E, Tsai WP, Harrod R, Fullen J, Tartaglia J, Franchini G. Vaccine. 2002 Aug 19;20(25-26):3171-86 (incorporated herein by reference); and

Catalytic single chain antibodies: Schultz, P.G. and Lerner, R.A., From molecular diversity to catalysis: lessons from the immune system 1995. Science 269, pp. 1835*1842 (incorporated herein by reference for its teaching of catalytic single chain antibodies responsible for the cleavage of protein substrates).

The capsids can also be assembled into empty particles by expression in mammalian, bacterial, fungal or insect cells. For example, AAV2 particles are known to be made from VP3 and VP2 capsid proteins in baculovirus. The same basic protocol can produce an empty AAAV particle comprising an AAAV capsid protein and also full particles.

The herein described recombinant AAAV nucleic acid derived vector can be encapsidated in an AAV particle. In particular, it can be encapsidated in an AAV1 particle, an AAV2 particle, an AAV3 particle, an AAV4 particle, an AAV5 particle, an AAV6 particle, and AAV7 particle or an AAV8 particle. A portion of any of the capsids, or a chimeric capsid particle as described above can be utilized, by standard methods using the appropriate capsid proteins in the encapsidation process, as long as the nucleic acid vector fits within the size limitation of the particle utilized. The encapsidation process itself is standard in the art. The AAAV replication machinery, i.e. the rep initiator proteins and other functions required for replication, can be utilized to produce the AAAV genome that can be packaged in an AAV1-8 capsid.

The recombinant AAAV virion containing a vector can also be produced by recombinant methods utilizing multiple plasmids. In one example, the AAAV rep nucleic acid would be cloned into one plasmid, the AAAV ITR nucleic acid would be cloned into another plasmid and the nucleic acid encoding a capsid (for example, an

AAV capsid from AAV1-AAV8) would be cloned on another plasmid. These plasmids would then be introduced into cells. The cells that were efficiently transduced by all three plasmids, would exhibit specific integration as well as the ability to produce AAAV recombinant virus. Additionally, two plasmids could be used where the AAAV rep nucleic acid would be cloned into one plasmid and the AAAV ITR and AAAV capsid would be cloned into another plasmid. These plasmids would then be introduced into cells. The cells that were efficiently transduced by both plasmids, would exhibit specific integration as well as the ability to produce AAAV recombinant virus.

The capsid proteins of the present invention can have about 70% homology, about 75% homology, 80% homology, 85% homology, 90% homology, 95% homology, 98% homology, 99% homology, or even 100% homology to the protein having the amino acid sequence encoded by the nucleotides set forth in SEQ ID NOS:10, 12 or 14. The percent homology used to identify proteins herein, can be based on a nucleotide-by-nucleotide comparison or more preferable is based on a computerized algorithm as described herein. Variations in the amino acid sequence of the AAAV capsid protein are contemplated herein, as long as the resulting particle comprising an AAAV capsid protein remains antigenically or immunologically distinct from AAV1-8 capsid, as can be routinely determined by standard methods. Specifically, for example, ELISA and Western blots can be used to determine whether a viral particle is antigenically or immunologically distinct from AAV2 or the other serotypes. Furthermore, the AAAV particle preferably retains tissue tropism distinction from AAV2, such as that exemplified in the examples herein. An AAAV chimeric particle comprising at least one AAAV coat protein may have a different tissue tropism from that of an AAAV particle consisting only of AAAV coat proteins, but is still distinct from the tropism of an AAV2 particle.

The invention further provides a recombinant AAAV virion, comprising an AAAV particle containing, *i.e.*, encapsidating, a vector comprising a pair of AAAV

inverted terminal repeats. The recombinant vector can further comprise an AAAV Rep-encoding nucleic acid. The vector encapsidated in the particle can further comprise an exogenous nucleic acid inserted between the inverted terminal repeats.

The invention further contemplates chimeric recombinant ITRs that contain a rep binding site and a TRS site recognized by that Rep protein. By "Rep protein" is meant all four of the Rep proteins, Rep 40, Rep 78, Rep 52, Rep 68. Alternatively, "Rep protein" could be one or more of the Rep proteins described herein. One example of a chimeric ITR would consist of an AAAV D region (SEQ ID NOs: 18, 19), an AAAV TRS site (SEQ ID NO: 20), an AAV2 hairpin and an AAV2 binding site. Another example would be an AAAV D region, an AAAV TRS site, an AAV3 hairpin and an AAV3 binding site. In these chimeric ITRs, the D region can be from AAV1-8. The hairpin can be derived from AAV 1-8. The binding site can be derived from any of AAV1-8. The D region and the TRS can be from the same serotype.

The chimeric ITRs can be combined with AAAV Rep protein and any of the AAV serotype capsids to obtain recombinant virion. For example, recombinant virion can be produced by an AAAV D region, an AAAV TRS site, an AAV2 hairpin, an AAV2 binding site, AAAV Rep protein and AAV1 capsid. This recombinant virion would possess the cellular tropism conferred by the AAV1 capsid protein and would possess the efficient replication conferred by the AAAV Rep.

Other examples of the ITR, Rep protein and Capsids that will produce recombinant virus are provided in the list below but not limited to :

AITR + ARep + ACap=virus
AITR + 5Rep + 1Cap=virus
AITR + 2Rep + 2Cap=virus
AITR + 7Rep + 3Cap=virus
AITR + 5Rep + 4Cap=virus

AITR + 5Rep + 6Cap=virus

1ITR + 1Rep + ACap=virus

2ITR + 2Rep + ACap=virus

4ITR + 4Rep + ACap=virus

5ITR + 5Rep + ACap=virus

6ITR + 6Rep + ACap=virus

(A= Avian, 1= AAV1, 2=AAV2, 3=AAV3, 4=AAV4, 5=AAV5, 6= AAV6)

In any of the constructs described herein, a promoter can be included. As used in the constructs herein, unless otherwise specified, Cap (capsid) refers to any of AAAV VP1, AAAV VP2, AAAV VP3, combinations thereof, functional fragments of any of VP1, VP2 or VP3, or chimeric capsids as described herein. The ITRs of the constructs described herein, can be chimeric recombinant ITRs as described elsewhere in the application.

Conjugates of recombinant or wild-type AAAV virions and nucleic acids or proteins can be used to deliver those molecules to a cell. For example, the purified AAAV can be used as a vehicle for delivering DNA bound to the exterior of the virus. Examples of this are to conjugate the DNA to the virion by a bridge using poly-L-lysine or another charged molecule. Also contemplated are virosomes that contain AAAV structural proteins (AAAV capsid proteins), lipids such as DOTAP, and nucleic acids that are complexed via charge interaction to introduce DNA into cells.

Also contemplated by this invention is a method of delivering a DNA vaccine to a cell, comprising: administering a liposome comprising DNA conjugated to an AAAV virion to a cell thus delivering the DNA vaccine to the cell.

High levels of humoral and cell-mediated immunity can be achieved via administration of DNA vaccines. Numerous studies have shown that immunization of

experimental animals with plasmid DNA encoding antigens from a wide spectrum of bacteria, viruses, protozoa and cancers leads to protective humoral and cell-mediated immunity (*Gregoriadis G.* "Genetic vaccines: strategies for optimization" Pharm Res. 15:661-70 (1998)).

Liposomes have been widely used to enhance the immune response. For example, a DNA vaccine constructed with the CMV promoter conjugated to env gp160 and rev genes has been shown to induce an effective immune response when inoculated via intramuscular, intraperitoneal, subcutaneous, intradermal and intranasal routes (*Fukushima I.N.* "Cationic liposomes are a strong adjuvant for a DNA vaccine of human immunodeficiency virus type 1" 13:1421-1428 (1997)). By immunizing with pCMV160/REV and cationic liposomes through various routes higher levels of both antibody production and delayed-type hypersensitivity were induced than by using DNA vaccine alone.

DNA vaccines can also be administered in combination with other agents in liposomes to increase levels of immunity. Co-administration of the DNA vaccine with IL-12 and granulocyte/macrophage CSF-expressing plasmids induced high levels of HIV-specific circulating T lymphocytes and in increase in delayed type hypersensitivity when administered by the intranasal route. The results indicate that intranasal administration of this DNA vaccine with liposomes, together with IL-12 and/or granulocyte/macrophage-CSF expressing plasmids, induces a strong level of anti-HIV-1 immune response (*Okada E.* "Intranasal immunization of a DNA vaccine with IL-12 and granulocyte-macrophage colony-stimulating factor (GM-CSF)-expressing plasmids in liposomes induces strong mucosal and cell-mediated immune responses against HIV-1 antigens" 159:3638-47 (1997)).

The liposome comprising a recombinant AAAV virion and a biological molecule or a DNA vaccine can be delivered to a specific cell type by covalently attaching a targeting moiety to a liposome or allowing the targeting moiety to become

integrated into the membrane as the liposome is formed. The targeting moiety can bind to a specific cell type, thus allowing the contents of the liposome to be delivered to a cell. For example, a targeting moiety specific for tumor cells can be incorporated into the liposome. Upon delivery of the liposome, the targeting moiety will bind to a tumor cell allowing thus allowing the toxin to enter the tumor cell. Alternatively, the targeting moiety can be a ligand that binds to a cell surface protein or receptor. Numerous cell-specific cell surface proteins are known which can be targeted by the present invention by incorporating a ligand for the cell surface protein into liposomes.

Also provided by this invention are conjugates that utilize the AAAV capsid or a unique region of the AAAV capsid protein (e.g. VP1, VP2 or VP3 or combinations thereof) to introduce DNA into cells. For example, the AAAV VP3 protein or fragment thereof, can be conjugated to a DNA on a plasmid that is conjugated to a lipid. Cells can be infected using the targeting ability of the VP3 capsid protein to achieve the desired tissue tropism, specific to AAAV. AAAV VP1 and VP2 proteins can also be utilized to introduce DNA or other molecules into cells. By further incorporating the Rep protein and the AAAV TRS into the DNA-containing conjugate, cells can be transduced and targeted integration can be achieved. For example, if AAAV specific targeted integration is desired, a conjugate composed of the AAAV VP3 capsid, AAAV rep or a fragment of AAAV rep, AAAV TRS, the rep binding site, the heterologous DNA of interest, and a lipid, can be utilized to achieve AAAV specific tropism and AAAV specific targeted integration in the genome.

Further provided by this invention are chimeric viruses where AAAV can be combined with herpes virus, baculovirus or other viruses to achieve a desired tropism associated with another virus. For example, the AAAV ITRs could be inserted in the herpes virus and cells could be infected. Post-infection, the ITRs of AAAV could be acted on by AAAV rep provided in the system or in a separate vehicle to rescue AAAV from the genome. Therefore, the cellular tropism of the herpes simplex virus can be combined with AAAV rep mediated targeted integration. Other viruses that could be

utilized to construct chimeric viruses include, lentivirus, retrovirus, pseudotyped retroviral vectors, and adenoviral vectors.

In another example, AAAV infects avian cells in much greater efficiencies than any other AAV. Traditionally, wild type AAAV has been propagated in chicken embryonated eggs in co-infection with avian adenoviruses (i.e., Fowl adenovirus type 1, better known as CELO virus). Recently, recombinant CELO virus that can replicate in chicken embryonated eggs has been constructed (Anne-Isabelle Michou et al, 1999, J virol. 73(2): 1399). A recombinant AAAV virion that encapsidates a therapeutic gene flanked by AAAV ITRs can be produced in embryonated chicken eggs upon co-infection with a recombinant CELO virus expressing the AAAV's rep and cap gene.

Any of the particles or virions comprising an exogenous nucleic acid encoding a protein described herein can be administered to a fertilized avian egg for the purposes of producing the recombinant protein in an avian egg. This is particularly useful for the production of vaccines as the protein produced in the avian egg can be readily purified by methods known in the art and administered to subjects in need of a vaccine.

The present invention further provides isolated nucleic acids of AAAV. For example, provided is an isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:1 (AAAV genome). This nucleic acid, or portions thereof, can be inserted into vectors, such as plasmids, yeast artificial chromosomes, or other viral vector (particle), if desired, by standard cloning methods. The present invention also provides an isolated nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO:1. The nucleotides of SEQ ID NO:1 can have minor modifications and still be contemplated by the present invention. For example, modifications that do not alter the amino acid encoded by any given codon (such as by modification of the third, "wobble," position in a codon) can readily be made, and such alterations are known in the art. Furthermore, modifications that cause a resulting neutral (conserved) amino acid substitution of a similar amino acid can be made in a coding region of the

genome. Additionally, modifications as described herein for the AAAV components, such as the ITRs, the p5 promoter, etc. are contemplated in this invention. Furthermore, modifications to regions of SEQ ID NO:1 other than in the ITR, TRS Rep binding site and hairpin are likely to be tolerated without serious impact on the function of the nucleic acid as a recombinant vector.

As used herein, the term "isolated" refers to a nucleic acid separated or significantly free from at least some of the other components of the naturally occurring organism, for example, the cell structural components or viral components commonly found associated with nucleic acids in the environment of the virus and/or other nucleic acids. The isolation of the native nucleic acids can be accomplished, for example, by techniques such as cell lysis followed by phenol plus chloroform extraction, followed by ethanol precipitation of the nucleic acids. The nucleic acids of this invention can be isolated from cells according to any of many methods well known in the art.

As used herein, the term "nucleic acid" refers to single-or multiple stranded molecules which may be DNA or RNA, or any combination thereof, including modifications to those nucleic acids. The nucleic acid may represent a coding strand or its complement, or any combination thereof. Nucleic acids may be identical in sequence to the sequences which are naturally occurring for any of the novel genes discussed herein or may include alternative codons which encode the same amino acid as those provided herein, including that which is found in the naturally occurring sequence. These nucleic acids can also be modified from their typical structure. Such modifications include, but are not limited to, methylated nucleic acids, the substitution of a non-bridging oxygen on the phosphate residue with either a sulfur (yielding phosphorothioate deoxynucleotides), selenium (yielding phosphorselenoate deoxynucleotides), or methyl groups (yielding methylphosphonate deoxynucleotides).

The present invention additionally provides an isolated nucleic acid that selectively hybridizes with any nucleic acid disclosed herein, including the entire AAAV genome and any unique fragment thereof, including the Rep and capsid

encoding sequences (e.g. SEQ ID NOS: 1, 2, 4, 6, 8, 10, 12, 14 and16-24). Specifically, the nucleic acid can selectively or specifically hybridize to an isolated nucleic acid consisting of the nucleotide sequence set forth in SEQ ID NO:1 (AAAV genome). The present invention further provides an isolated nucleic acid that selectively or specifically hybridizes with an isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:1 (AAAV genome). By "selectively hybridizes" as used herein is meant a nucleic acid that hybridizes to one of the disclosed nucleic acids under sufficient stringency conditions without significant hybridization to a nucleic acid encoding an unrelated protein, and particularly, without detectably hybridizing to nucleic acids of AAV2 or other AAVs. Thus, a nucleic acid that selectively hybridizes with a nucleic acid of the present invention will not selectively hybridize under stringent conditions with a nucleic acid encoding a different protein or the corresponding protein from a different serotype of the virus, and vice versa. A "specifically hybridizing" nucleic acid is one that hybridizes under stringent conditions to only a nucleic acid found in AAAV. Therefore, nucleic acids for use, for example, as primers and probes to detect or amplify the target nucleic acids are contemplated herein. Nucleic acid fragments that selectively hybridize to any given nucleic acid can be used, *e.g.*, as primers and or probes for further hybridization or for amplification methods (*e.g.*, polymerase chain reaction (PCR), ligase chain reaction (LCR)). Additionally, for example, a primer or probe can be designed that selectively hybridizes with both AAAV and a gene of interest carried within the AAAV vector (*i.e.*, a chimeric nucleic acid).

Stringency of hybridization is controlled by both temperature and salt concentration of either or both of the hybridization and washing steps. Typically, the stringency of hybridization to achieve selective hybridization involves hybridization in high ionic strength solution (6X SSC or 6X SSPE) at a temperature that is about 12-25°C below the T_m (the melting temperature at which half of the molecules dissociate from their hybridization partners) followed by washing at a combination of temperature and salt concentration chosen so that the washing temperature is about 5°C to 20°C

below the T_m. The temperature and salt conditions are readily determined empirically in preliminary experiments in which samples of reference DNA immobilized on filters are hybridized to a labeled nucleic acid of interest and then washed under conditions of different stringencies. Hybridization temperatures are typically higher for DNA-RNA and RNA-RNA hybridizations. The washing temperatures can be used as described above to achieve selective stringency, as is known in the art. (Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989; Kunkel et al. *Methods Enzymol.* 1987:154:367, 1987). For the nucleic acids of the present invention, stringent hybridization conditions for a DNA:DNA hybridization can be at about 65°C (in aqueous solution) in 6X SSC or 6X SSPE followed by washing at 65°C. Therefore, the present invention provides nucleic acids that selectively hybridize to any of the nucleic acids described herein at about 65°C (in aqueous solution) in 6X SSC or 6X SSPE followed by washing at 65°C. Stringency of hybridization and washing, if desired, can be reduced accordingly as the degree of complementarity desired is decreased, and further, depending upon the G-C or A-T richness of any area wherein variability is searched for. Likewise, stringency of hybridization and washing, if desired, can be increased accordingly as homology desired is increased, and further, depending upon the G-C or A-T richness of any area wherein high homology is desired, all as known in the art.

A nucleic acid that selectively hybridizes to any portion of the AAAV genome is contemplated herein. Therefore, a nucleic acid that selectively hybridizes to AAAV can be of longer length than the AAAV genome, it can be about the same length as the AAAV genome or it can be shorter than the AAAV genome. The length of the nucleic acid is limited on the shorter end of the size range only by its specificity for hybridization to AAAV, *i.e.*, once it is too short, typically less than about 5 to 7 nucleotides in length, it will no longer bind specifically to AAAV, but rather will hybridize to numerous background nucleic acids. Additionally contemplated by this invention is a nucleic acid that has a portion that specifically hybridizes to AAAV and a portion that specifically hybridizes to a gene of interest inserted within AAAV.

The present invention further provides an isolated nucleic acid encoding an avian adeno-associated virus Rep protein. The AAAV Rep proteins are encoded by open reading frame (ORF) 1 of the AAAV genome. Examples of the AAAV Rep genes are shown in the nucleic acid set forth in SEQ ID NO:1, and include nucleic acids consisting essentially of the nucleotide sequences set forth in SEQ ID NOS:4 (Rep52), 2 (Rep78), 8 (Rep40), and 6 (Rep68), and nucleic acids comprising the nucleotide sequences set forth in SEQ ID NOS:2, 4, 6 and 8. Also contemplated herein are vectors comprising nucleotides 1-600 of SEQ ID NO: 1 which encode the first 200 amino acids of Rep. However, the present invention contemplates that the Rep nucleic acid can include any one, two, three, or four of the four Rep proteins, in any order, in such a nucleic acid. Furthermore, minor modifications are contemplated in the nucleic acid, such as silent mutations in the coding sequences, mutations that make neutral or conservative changes in the encoded amino acid sequence, and mutations in regulatory regions that do not disrupt the expression of the gene. Examples of other minor modifications are known in the art. Further modifications can be made in the nucleic acid, such as to disrupt or alter expression of one or more of the Rep proteins in order to, for example, determine the effect of such a disruption; such as to mutate one or more of the Rep proteins to determine the resulting effect, etc. However, in general, a modified nucleic acid encoding a Rep protein will have at least about 70%, about 75%, about 80%, about 85%, about 90%, about 93%, about 95%, about 98% or 100% homology to the Rep nucleic sequences described herein e.g., SEQ ID NOS: 2, 4, 6 and 8, and the Rep polypeptide encoded therein will have overall about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 98%, about 99% or 100% homology with the amino acid sequence described herein, e.g., SEQ ID NOS:3, 5, 7 and 9. Percent homology is determined by the techniques described herein.

The present invention also provides an isolated nucleic acid that selectively or specifically hybridizes with a nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NOS: 2, 4, 6 and 8 and an isolated nucleic acid that

selectively hybridizes with a nucleic acid comprising the nucleotide sequence set forth in SEQ ID NOS: 2, 4, 6 and 8. "Selectively hybridizing" and "stringency of hybridization" is defined elsewhere herein.

As described above, the present invention provides the nucleic acid encoding a Rep 40 protein and, in particular an isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO: 8, an isolated nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO: 8, and a nucleic acid encoding the avian adeno-associated virus protein having the amino acid sequence set forth in SEQ ID NO: 9. The present invention also provides the nucleic acid encoding a Rep 52 protein, and in particular an isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:4, an isolated nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO:4, and a nucleic acid encoding the avian adeno-associated virus Rep protein having the amino acid sequence set forth in SEQ ID NO:5. The present invention further provides the nucleic acid encoding a Rep 68 protein and, in particular an isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO: 6, an isolated nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO: 6, and a nucleic acid encoding the avian adeno-associated virus protein having the amino acid sequence set forth in SEQ ID NO: 7. And, further, the present invention provides the nucleic acid encoding a Rep 78 protein, and in particular an isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:2, an isolated nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO:, and a nucleic acid encoding the avian adeno-associated virus Rep protein having the amino acid sequence set forth in SEQ ID NO:3. As described elsewhere herein, these nucleic acids can have minor modifications, including silent nucleotide substitutions, mutations causing conservative amino acid substitutions in the encoded proteins, and mutations in control regions that do not or minimally affect the encoded amino acid sequence.

The present invention further provides a nucleic acid encoding the entire AAAV Capsid polypeptide. Furthermore, the present invention provides a nucleic acid encoding each of the three AAAV coat proteins, VP1, VP2, and VP3. Thus, the present invention provides a nucleic acid encoding AAAV VP1, a nucleic acid encoding AAAV VP2, and a nucleic acid encoding AAAV VP3. Thus, the present invention provides a nucleic acid encoding the amino acid sequence set forth in SEQ ID NO:11 (VP1); a nucleic acid encoding the amino acid sequence set forth in SEQ ID NO:13 (VP2), and a nucleic acid encoding the amino acid sequence set forth in SEQ ID NO:15 (VP3). The present invention also specifically provides a nucleic acid comprising SEQ ID NO:10 (VP1 gene); a nucleic acid comprising SEQ ID NO:12 (VP2 gene); and a nucleic acid comprising SEQ ID NO:14 (VP3 gene). The present invention also specifically provides a nucleic acid consisting essentially of SEQ ID NO:10 (VP1 gene), a nucleic acid consisting essentially of SEQ ID NO:12 (VP2 gene), and a nucleic acid consisting essentially of SEQ ID NO:14 (VP3 gene). The present invention also provides a nucleic acid comprising nucleotides 1347-2127 of SEQ ID NO: 10 (encoding amino acids 449-709 of VP1). Minor modifications in the nucleotide sequences encoding the capsid, or coat, proteins are contemplated, as described above for other AAAV nucleic acids. However, in general, a modified nucleic acid encoding a capsid protein will have at least about 70%, about 75%, about 80%, about 85%, about 90%, about 93%, about 95%, about 98% or 100% homology to the capsid nucleic acid sequences described herein e.g., SEQ ID NOS: 10, 12, and 14, and the capsid polypeptide encoded therein will have overall about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 98%, about 99% or 100% homology with the amino acid sequence described herein, e.g., SEQ ID NOS:11, 13, and 15. Nucleic acids that selectively hybridize with the nucleic acids of SEQ ID NOS: 10, 12, and 14 under the conditions described above are also provided.

The present invention also provides a cell containing one or more of the herein described nucleic acids, such as the AAAV genome, AAAV ORF1 and ORF2, each AAAV Rep protein gene, or each AAAV capsid protein gene. Such a cell can be any

desired cell and can be selected based upon the use intended. For example, cells can include bacterial cells, yeast cells, insect cells, human HeLa cells and simian Cos cells as well as other human and mammalian cells and cell lines. Primary cultures as well as established cultures and cell lines can be used. Nucleic acids of the present invention can be delivered into cells by any selected means, in particular depending upon the target cells. Many delivery means are well-known in the art. For example, electroporation, calcium phosphate precipitation, microinjection, cationic or anionic liposomes, and liposomes in combination with a nuclear localization signal peptide for delivery to the nucleus can be utilized, as is known in the art. Additionally, if the nucleic acids are in a viral particle, the cells can simply be transduced with the virion by standard means known in the art for AAV transduction. Small amounts of the recombinant AAAV virus can be made to infect cells and produce more of itself.

The invention provides purified AAAV polypeptides. The term "polypeptide" as used herein refers to a polymer of amino acids and includes full-length proteins and fragments thereof. Thus, "protein," polypeptide," and "peptide" are often used interchangeably herein. Substitutions can be selected by known parameters to be neutral (*see, e.g.,* Robinson WE Jr, and Mitchell WM., AIDS 4:S151-S162 (1990)). As will be appreciated by those skilled in the art, the invention also includes those polypeptides having slight variations in amino acid sequences or other properties. Such variations may arise naturally as allelic variations (*e.g.,* due to genetic polymorphism) or may be produced by human intervention (*e.g.,* by mutagenesis of cloned DNA sequences), such as induced point, deletion, insertion and substitution mutants. Minor changes in amino acid sequence are generally preferred, such as conservative amino acid replacements, small internal deletions or insertions, and additions or deletions at the ends of the molecules. Substitutions may be designed based on, for example, the model of Dayhoff, *et al.* (in *Atlas of Protein Sequence and Structure 1978*, Nat'l Biomed. Res. Found., Washington, D.C.). These modifications can result in changes in the amino acid sequence, provide silent mutations, modify a restriction site, or provide other specific mutations. The location of any modifications to the polypeptide will

often determine its impact on function. Particularly, alterations in regions non-essential to protein function will be tolerated with fewer effects on function. Elsewhere in the application regions of the AAAV proteins are described to provide guidance as to where substitutions, additions or deletions can be made to minimize the likelihood of disturbing the function of the variant.

A polypeptide of the present invention can be readily obtained by any of several means. For example, the polypeptide of interest can be synthesized chemically by standard methods. Additionally, the coding regions of the genes can be recombinantly expressed and the resulting polypeptide isolated by standard methods. Furthermore, an antibody specific for the resulting polypeptide can be raised by standard methods (see, *e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1988), and the protein can be isolated from a cell expressing the nucleic acid encoding the polypeptide by selective hybridization with the antibody. This protein can be purified to the extent desired by standard methods of protein purification (see, *e.g.*, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989).

Typically, to be unique, a polypeptide fragment of the present invention will be at least about 5 amino acids in length; however, unique fragments can be 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids in length. A unique polypeptide will typically comprise such a unique fragment; however, a unique polypeptide can also be determined by its overall homology. A unique polypeptide can be 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids in length. Uniqueness of a polypeptide fragment can readily be determined by standard methods such as searches of computer databases of known peptide or nucleic acid sequences or by hybridization studies to the nucleic acid encoding the protein or to the protein itself, as known in the art. The uniqueness of a polypeptide fragment can also be determined immunologically

as well as functionally. Uniqueness can be simply determined in an amino acid-by-amino acid comparison of the polypeptides.

An antigenic or immunoreactive fragment of this invention is typically an amino acid sequence of at least about 5 consecutive amino acids, and it can be derived from the AAAV polypeptide amino acid sequence. An antigenic AAAV fragment is any fragment unique to the AAAV protein, as described herein, against which an AAAV-specific antibody can be raised, by standard methods. Thus, the resulting antibody-antigen reaction should be specific for AAAV.

The present invention provides an isolated AAAV Rep protein. An AAAV Rep polypeptide is encoded by ORF1 of AAAV. The present invention also provides each individual AAAV Rep protein. Thus the present invention provides AAAV Rep 40 (e.g., SEQ ID NO: 9), or a unique fragment thereof. The present invention provides AAAV Rep 52 (e.g., SEQ ID NO: 5), or a unique fragment thereof. The present invention provides AAAV Rep 68 (e.g., SEQ ID NO: 7), or a unique fragment thereof. The present invention provides an example of AAAV Rep 78 (e.g., SEQ ID NO: 3), or a unique fragment thereof. By "unique fragment thereof" is meant any smaller polypeptide fragment encoded by an AAAV rep gene that is of sufficient length to be found only in the Rep polypeptide. Substitutions and modifications of the amino acid sequence can be made as described above and, further, can include protein processing modifications, such as glycosylation, to the polypeptide.

The present invention further provides an AAAV Capsid polypeptide or a unique fragment thereof. AAAV capsid polypeptide is encoded by ORF 2 of AAAV. The present invention further provides the individual AAAV capsid proteins, VP1, VP2 and VP3 or unique fragments thereof. Thus, the present invention provides an isolated polypeptide having the amino acid sequence set forth in SEQ ID NO:11 (VP1). The present invention additionally provides an isolated polypeptide having the amino acid sequence set forth in SEQ ID NO:13 (VP2). The present invention also provides

an isolated polypeptide having the amino acid sequence set forth in SEQ ID NO:15 (VP3). By "unique fragment thereof" is meant any smaller polypeptide fragment encoded by any AAAV capsid gene that is of sufficient length to be found only in the AAAV capsid protein. Substitutions and modifications of the amino acid sequence can be made as described above and, further, can include protein processing modifications, such as glycosylation, to the polypeptide. However, an AAAV Capsid polypeptide including all three coat proteins will have greater than about 56% overall homology to the polypeptide encoded by the nucleotides set forth in SEQ ID NOS:10, 12 or 14. The protein can have about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, 93%, 95%, 97% or even 100% homology to the amino acid sequence encoded by the nucleotides set forth in SEQ ID NOS: 10, 12 or 14. An AAAV VP1 polypeptide can have at least about 58%, about 60%, about 70%, about 80%, about 90%, 93%, 95%, 97% or about 100% homology to the amino acid sequence set forth in SEQ ID NO:10. An AAAV VP2 polypeptide can have at least about 58%, about 60%, about 70%, about 80%, about 90%, 93%, 95%, 97% or about 100% homology to the amino acid sequence set forth in SEQ ID NO:12. An AAAV VP3 polypeptide can have at least about 60%, about 70%, about 80%, about 90%, 93%, 95%, 97% or about 100% homology to the amino acid sequence set forth in SEQ ID NO:14.

The present invention further provides an isolated antibody that specifically binds an AAAV Rep protein or a unique epitope thereof. Also provided are isolated antibodies that specifically bind the AAAV Rep 52 protein, the AAAV Rep 40 protein, the AAAV Rep 68 protein and the AAAV Rep 78 protein having the amino acid sequences set forth in SEQ ID NO:5, SEQ ID NO: 9, SEQ ID NO: 7 and SEQ ID NO: 3, respectively or that specifically binds a unique fragment thereof. Clearly, any given antibody can recognize and bind one of a number of possible epitopes present in the polypeptide; thus only a unique portion of a polypeptide (having the epitope) may need to be present in an assay to determine if the antibody specifically binds the polypeptide.

The present invention additionally provides an isolated antibody that specifically binds any of the avian adeno-associated virus Capsid proteins (VP1, VP2 or VP3), a unique epitope thereof, or the polypeptide comprising all three AAAV coat proteins. Also provided is an isolated antibody that specifically binds the AAAV capsid protein having the amino acid sequence set forth in SEQ ID NO:11 (VP1), or that specifically binds a unique fragment thereof. The present invention further provides an isolated antibody that specifically binds the AAAV Capsid protein having the amino acid sequence set forth in SEQ ID NO:13 (VP2), or that specifically binds a unique fragment thereof. The invention additionally provides an isolated antibody that specifically binds the AAAV Capsid protein having the amino acid sequence set forth in SEQ ID NO:15 (VP3), or that specifically binds a unique fragment thereof. Again, any given antibody can recognize and bind one of a number of possible epitopes present in the polypeptide; thus only a unique portion of a polypeptide (having the epitope) may need to be present in an assay to determine if the antibody specifically binds the polypeptide.

The antibody can be a component of a composition that comprises an antibody that specifically binds the AAAV protein. The composition can further comprise, *e.g.*, serum, serum-free medium, or a pharmaceutically acceptable carrier such as physiological saline, etc..

By "an antibody that specifically binds" an AAAV polypeptide or protein is meant an antibody that selectively binds to an epitope on any portion of the AAAV peptide such that the antibody binds specifically to the corresponding AAAV polypeptide without significant background. Specific binding by an antibody further means that the antibody can be used to selectively remove the target polypeptide from a sample comprising the polypeptide or and can readily be determined by radioimmunoassay (RIA), bioassay, or enzyme-linked immunosorbant (ELISA) technology. An ELISA method effective for the detection of the specific antibody-antigen binding can, for example, be as follows: (1) bind the antibody to a substrate;

(2) contact the bound antibody with a sample containing the antigen; (3) contact the above with a secondary antibody bound to a detectable moiety (e.g., horseradish peroxidase enzyme or alkaline phosphatase enzyme); (4) contact the above with the substrate for the enzyme; (5) contact the above with a color reagent; (6) observe the color change.

An antibody can include antibody fragments such as Fab fragments which retain the binding activity. Antibodies can be made as described in, *e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1988). Briefly, purified antigen can be injected into an animal in an amount and in intervals sufficient to elicit an immune response. Antibodies can either be purified directly, or spleen cells can be obtained from the animal. The cells are then fused with an immortal cell line and screened for antibody secretion. Individual hybridomas are then propagated as individual clones serving as a source for a particular monoclonal antibody.

The present invention additionally provides a method of screening a cell for infectivity by AAAV comprising contacting the cell with AAAV and detecting the presence of AAAV in the cells. AAAV particles can be detected using any standard physical or biochemical methods.

The present invention provides a method of screening for cells that are permissive to AAAV infection comprising identifying the presence of N-linked terminal lactose on the surface of a cell, contacting the N-linked terminal lactose containing cell with AAAV and detecting the presence of AAAV virus in the cell, whereby if AAAV virus is detected in the cells, the N-linked terminal lactose containing cell is permissive to AAAV infection. In one example of such a method, based on the teaching in the Examples, uses *Erythrina corralodendron* lectin to detect cells that would allow efficient binding of AAAV and possible transduction. A closely related method using sialic acid binding lectins to screen for AAV5 transduction is

described in Walters et al. (Adeno-associated virus serotype 4 (AAV4) and AAV5 both require sialic acid binding for hemagglutination and efficient transduction but differ in sialic acid linkage specificity, J Virol. 2001 Aug; 75(15):6884-93, incorporated herein by reference).

For the screening methods of the present invention, monoclonal antibodies to different forms of conjugated lactose can be produced (Sato et al JBC 2000. May 19;275(20):15422-31). Cells are contacted with these antibodies to select cells that contain the appropriate N-linked terminal lactose. A number of antibodies exist which bind specific lactose conjugates and can be used to screen for N-linked terminal lactose containing cells. These antibodies can be fluorescently labeled and used in situ. Alternatively, antibodies can be bound to a plate and target cells added. The wells are then washed and cells that express the antigen will bind to the N-linked terminal lactose antibody. Cells that bind to the sialic acid can be visualized by staining. Another way to screen for permissive cells is to chemically remove the glycans from the cell surface and fractionate these by thin layer chromatography. The presence of the correct form of N-linked terminal lactose can be confirmed by hybridizing the blot with labeled virus. Free virus is washed off and the specifically bound virus visualized by detecting the label. Alternatively, whole membrane proteins could be used and separated by PAGE, transferred to a membrane and probed as described above.

Additionally, physical methods that can be used for this detection include DNA based methods such as 1) polymerase chain reaction (PCR) for viral DNA or RNA or 2) direct hybridization with labeled probes, and immunological methods such as by 3) antibody directed against the viral structural or non- structural proteins. Catalytic methods of viral detection include, but are not limited to, detection of site and strand specific DNA nicking activity of Rep proteins or replication of an AAV origin-containing substrate. Reporter genes can also be utilized to detect cells that transduce AAAV. For example, β-gal, green fluorescent protein or luciferase can be inserted into a recombinant AAAV. The cell can then be contacted with the recombinant AAAV,

either *in vitro* or *in vivo* and a colorimetric assay could detect a color change in the cells that would indicate transduction of AAAV in the cell. Additional detection methods are outlined in Fields, *Virology*, Raven Press, New York, New York. 1996.

For screening a cell for infectivity by AAAV, wherein the presence of AAAV in the cells is determined by nucleic acid hybridization methods, a nucleic acid probe for such detection can comprise, for example, a unique fragment of any of the AAAV nucleic acids provided herein. The uniqueness of any nucleic acid probe can readily be determined as described herein. Additionally, the presence of AAAV in cells can be determined by fluorescence, antibodies to gene products, focus forming assays, plaque lifts, Western blots and chromogenic assays. The nucleic acid can be, for example, the nucleic acid whose nucleotide sequence is set forth in SEQ ID NO: 1, 2, 4, 6, 8, 10, 12, 14, and 16-24 or a unique fragment thereof.

The present invention includes a method of determining the suitability of an AAAV vector for administration to a subject comprising administering to an antibody-containing sample from the subject an antigenic fragment of an isolated AAAV Rep or Capsid protein, and detecting neutralizing antibody-antigen reaction in the sample, the presence of a neutralizing reaction indicating the AAAV vector may be unsuitable for use in the subject. The present method of determining the suitability of an AAAV vector for administration to a subject can comprise contacting an antibody-containing sample from the subject with a unique antigenic or immunogenic fragment of an AAAV Rep protein (e.g. Rep 40, Rep 52, Rep 68, Rep 78) and detecting an antibody-antigen reaction in the sample, the presence of a reaction indicating the AAAV vector to be unsuitable for use in the subject. The AAAV Rep proteins are provided herein, and their antigenic fragments are routinely determined. The AAAV capsid protein can be used to select an antigenic or immunogenic fragment, for example from the amino acid sequence set forth in SEQ ID NO:11 (VP1), the amino acid sequence set forth in SEQ ID NO: 13 (VP2) or the amino acid sequence set forth in SEQ ID NO:15 (VP3). Alternatively, or additionally, an antigenic or immunogenic fragment of an isolated

AAAV Rep protein can be utilized in this determination method. The AAAV Rep protein from which an antigenic fragment is selected can have the amino acid sequence encoded by the nucleic acid set forth in SEQ ID NO:1, the amino acid sequence set forth in SEQ ID NO:3, or the amino acid sequence set forth in SEQ ID NO:5, the amino acid sequence set forth in SEQ ID NO: 7, or the amino acid sequence set forth in SEQ ID NO:9.

The AAAV polypeptide fragments can be analyzed to determine their antigenicity, immunogenicity and/or specificity. Briefly, various concentrations of a putative immunogenically specific fragment are prepared and administered to a subject and the immunological response (e.g., the production of antibodies or cell mediated immunity) of an animal to each concentration is determined. The amounts of antigen administered depend on the subject, e.g. a human, rabbit or a guinea pig, the condition of the subject, the size of the subject, etc. Thereafter an animal so inoculated with the antigen can be exposed to the AAAV viral particle or AAAV protein to test the immunoreactivity or the antigenicity of the specific immunogenic fragment. The specificity of a putative antigenic or immunogenic fragment can be ascertained by testing sera, other fluids or lymphocytes from the inoculated animal for cross reactivity with other closely related viruses, such as AAV1-8.

By the "suitability of an AAAV vector for administration to a subject" is meant a determination of whether the AAAV vector will elicit a neutralizing immune response upon administration to a particular subject. A vector that does not elicit a significant immune response is a potentially suitable vector, whereas a vector that elicits a significant, neutralizing immune response (e.g. at least 90%) is thus likely to be unsuitable for use in that subject. Significance of any detectable immune response is a standard parameter understood by the skilled artisan in the field. For example, one can incubate the subject's serum with the virus, then determine whether that virus retains its ability to transduce cells in culture. If such virus cannot transduce cells in culture, the vector likely has elicited a significant immune response.

Alternatively, or additionally, one skilled in the art could determine whether or not AAAV administration would be suitable for a particular cell type of a subject. For example, the artisan could culture muscle cells *in vitro* and transduce the cells with AAAV in the presence or absence of the subject's serum. If there is a reduction in transduction efficiency, this could indicate the presence of a neutralizing antibody or other factors that may inhibit transduction. Normally, greater than 90% inhibition would have to be observed in order to rule out the use of AAAV as a vector. However, this limitation could be overcome by treating the subject with an immunosuppressant that could block the factors inhibiting transduction.

As will be recognized by those skilled in the art, numerous types of immunoassays are available for use in the present invention to detect binding between an antibody and an AAAV polypeptide of this invention. For instance, direct and indirect binding assays, competitive assays, sandwich assays, and the like, as are generally described in, *e.g.*, U.S. Pat. Nos. 4,642,285; 4,376,110; 4,016,043; 3,879,262; 3,852,157; 3,850,752; 3,839,153; 3,791,932; and Harlow and Lane, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, N.Y. (1988). For example, enzyme immunoassays such as immunofluorescence assays (IFA), enzyme linked immunosorbent assays (ELISA) and immunoblotting can be readily adapted to accomplish the detection of the antibody. An ELISA method effective for the detection of the antibody bound to the antigen can, for example, be as follows: (1) bind the antigen to a substrate; (2) contact the bound antigen with a fluid or tissue sample containing the antibody; (3) contact the above with a secondary antibody specific for the antigen and bound to a detectable moiety (e.g., horseradish peroxidase enzyme or alkaline phosphatase enzyme); (4) contact the above with the substrate for the enzyme; (5) contact the above with a color reagent; (6) observe color change.

The antibody-containing sample of this method can comprise any biological sample which would contain the antibody or a cell containing the antibody, such as blood, plasma, serum, bone marrow, saliva, urine and mucus.

The present invention also provides a method of producing the AAAV virus by transducing a cell with the nucleic acid encoding the virus. The present invention also provides AAAV produced by the method of transducing a cell with the nucleic acid encoding the virus.

The present method further provides a method of delivering an exogenous (heterologous) nucleic acid to a cell comprising administering to the cell an AAAV particle containing a vector comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, thereby delivering the nucleic acid to the cell.

The AAV ITRs in the vector for the herein described delivery methods can be AAAV ITRs (SEQ ID NOS: 16 and 17). Furthermore, the AAV ITRs in the vector for the herein described nucleic acid delivery methods can also comprise AAV1-8 inverted terminal repeats.

The present invention also includes a method of delivering a heterologous nucleic acid to a subject comprising administering to a cell from the subject an AAAV particle containing a vector comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, and returning the cell to the subject, thereby delivering the nucleic acid to the subject. The AAV ITRs can be any AAV ITRs, including AAAV ITRs, AAV5 ITRs and AAV2 ITRs.. For example, in an *ex vivo* administration, cells are isolated from a subject by standard means according to the cell type and placed in appropriate culture medium, again according to cell type (*see, e.g.*, ATCC catalog). Viral particles are then contacted with the cells as described above, and the virus is allowed to transduce the cells. Cells can then be transplanted back into the subject's body, again by means standard for the cell type and tissue (*e. g.*, in general, U.S. Patent

No. 5,399,346; for neural cells, Dunnett, S.B. and Björklund, A., eds., *Transplantation: Neural Transplantation-A Practical Approach,* Oxford University Press, Oxford (1992)). If desired, prior to transplantation, the cells can be studied for degree of transduction by the virus, by known detection means and as described herein. Cells for *ex vivo* transduction followed by transplantation into a subject can be selected from those listed above, or can be any other selected cell. Preferably, a selected cell type is examined for its capability to be transfected by AAAV. Preferably, the selected cell will be a cell readily transduced with AAAV particles; however, depending upon the application, even cells with relatively low transduction efficiencies can be useful, particularly if the cell is from a tissue or organ in which even production of a small amount of the protein or antisense RNA encoded by the vector will be beneficial to the subject.

The present invention further provides a method of delivering a nucleic acid to a cell in a subject comprising administering to the subject an AAAV particle containing a vector comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, thereby delivering the nucleic acid to a cell in the subject. Administration can be an *ex vivo* administration directly to a cell removed from a subject, such as any of the cells listed above, followed by replacement of the cell back into the subject, or administration can be *in vivo* administration to a cell in the subject. For *ex vivo* administration, cells are isolated from a subject by standard means according to the cell type and placed in appropriate culture medium, again according to cell type (*see, e.g.*, ATCC catalog). Viral particles are then contacted with the cells as described above, and the virus is allowed to transfect the cells. Cells can then be transplanted back into the subject's body, again by means standard for the cell type and tissue (*e. g.*, for neural cells, Dunnett, S.B. and Björklund, A., eds., *Transplantation: Neural Transplantation-A Practical Approach,* Oxford University Press, Oxford (1992)). If desired, prior to transplantation, the cells can be studied for degree of transfection by the virus, by known detection means and as described herein.

The present invention further provides a method of delivering a nucleic acid to a cell in a subject having neutralizing antibodies to AAV1-8 comprising administering to the subject an AAAV particle containing a vector comprising the nucleic acid, thereby delivering the nucleic acid to a cell in the subject. A subject that has neutralizing antibodies to AAV1-8 can readily be determined by any of several known means, such as contacting AAV1-8 protein(s) with an antibody-containing sample, such as blood, from a subject and detecting an antigen-antibody reaction in the sample. Delivery of the AAV1-8 particle can be by either *ex vivo* or *in vivo* administration as herein described. Thus, a subject who might have an adverse immunogenic reaction to a vector administered in an AAV2 viral particle can have a desired nucleic acid delivered using an AAV1-8 particle. This delivery system can be particularly useful for subjects who have received therapy utilizing AAV1-8 particles in the past and have developed antibodies to AAV1-8. An AAAV regimen can now be substituted to deliver the desired nucleic acid.

In any of the methods of delivering heterologous nucleic acids to a cell or subject described herein, the AAAV-conjugated nucleic acid or AAAV particle-conjugated nucleic acids described herein can be used.

In vivo administration to a human subject or an animal model can be by any of many standard means for administering viruses, depending upon the target organ, tissue or cell. Virus particles can be administered orally, parenterally (*e.g.*, intravenously), by intramuscular injection, by direct tissue or organ injection, by intraperitoneal injection, topically, transdermally, via aerosol delivery, via the mucosa or the like. Viral nucleic acids (non-encapsidated) can also be administered, *e.g.*, as a complex with cationic liposomes, or encapsulated in anionic liposomes. The present compositions can include various amounts of the selected viral particle or non-encapsidated viral nucleic acid in combination with a pharmaceutically acceptable carrier and, in addition, if desired, may include other medicinal agents, pharmaceutical agents, carriers, adjuvants, diluents, etc. Parental administration, if used, is generally characterized by injection. Injectables can

be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Dosages will depend upon the mode of administration, the disease or condition to be treated, and the individual subject's condition, but will be that dosage typical for and used in administration of other AAV vectors, such as AAV2 vectors. Often a single dose can be sufficient; however, the dose can be repeated if desirable.

Administration methods can be used to treat brain disorders such as Parkinson's disease, Alzheimer's disease, and demyelination disease. Other diseases that can be treated by these methods include metabolic disorders such as , muscoloskeletal diseases, cardiovascular disease, cancer, and autoimmune disorders.

Administration of this recombinant AAAV virion to the cell can be accomplished by any means, including simply contacting the particle, optionally contained in a desired liquid such as tissue culture medium, or a buffered saline solution, with the cells. The virion can be allowed to remain in contact with the cells for any desired length of time, and typically the virion is administered and allowed to remain indefinitely. For such *in vitro* methods, the virion can be administered to the cell by standard viral transduction methods, as known in the art and as exemplified herein. Titers of virus to administer can vary, particularly depending upon the cell type, but will be typical of that used for AAV transduction in general which is well known in the art. Additionally the titers used to transduce the particular cells in the present examples can be utilized.

The cells that can be transduced by the present recombinant AAAV virion can include any desired cell, such as the following cells and cells derived from the following tissues, human as well as other mammalian tissues, such as primate, horse, sheep, goat, pig, dog, rat, and mouse and avian species: Adipocytes, Adenocyte, Adrenal cortex, Amnion, Aorta, Ascites, Astrocyte, Bladder, Bone, Bone marrow, Brain, Breast, Bronchus, Cardiac muscle, Cecum, Cervix, Chorion, Colon, Conjunctiva,

Connective tissue, Cornea, Dermis, Duodenum, Endometrium, Endothelium, Endothelial cells, Epithelial tissue, Epithelial cells, Epidermis, Esophagus, Eye, Fascia, Fibroblasts, Foreskin, Gastric, Glial cells, Glioblast, Gonad, Hepatic cells, Histocyte, Ileum, Intestine, small Intestine, Jejunum, Keratinocytes, Kidney, Larynx, Leukocytes, Lipocyte, Liver, Lung, Lymph node, Lymphoblast, Lymphocytes, Macrophages, Mammary alveolar nodule, Mammary gland, Mastocyte, Maxilla, Melanocytes, Mesenchymal, Monocytes, Mouth, Myelin, Myoblasts Nervous tissue, Neuroblast, Neurons, Neuroglia, Osteoblasts, Osteogenic cells, Ovary, Palate, Pancreas, Papilloma, Peritoneum, Pituicytes, Pharynx, Placenta, Plasma cells, Pleura, Prostate, Rectum, Salivary gland, Skeletal muscle, Skin, Smooth muscle, Somatic, Spleen, Squamous, Stomach, Submandibular gland, Submaxillary gland, Synoviocytes, Testis, Thymus, Thyroid, Trabeculae, Trachea, Turbinate, Umbilical cord, Ureter, and Uterus.

The methods of the present invention are also useful for the delivery of AAAV vectors that express ribozymes or small interfering RNAs (siRNAs). Both methods can reduce protein expression by minimizing or completely abolishing mRNA levels of targeted genes. Applications in the poultry industry are also contemplated. These include delivery of a ribozyme or siRNA against chicken myostatin, a gene controlling muscle mass. In addition, the ability of AAAV vectors to deliver genes to a variety of tissues to express genetic information effectively for long periods of time, and to have a good safety profile make avian AAVs an attractive vector for genetic immunization of chickens. Avian AAV vectors could be used for *in ovo* or post-hatch vaccination of chickens against diseases such as Marek's, coccidiosis, Newcastle disease, etc.

Also, a hallmark of avian AAV infection is the inhibition of avian viruses during co-infection. The present invention shows that this is a function of the avian AAV non-structural proteins. Incorporation of these sequences into a viral vector or addition of recombinant protein to eggs could be used as a method to inhibit viral infection and promote growth/development.

A method of blocking AAAV infection is provided. The method is based on the findings in the Examples that AAAV requires N-linked terminal lactose present on cell surface proteins for efficient binding and entry. Thus, lactose conjugates, dendrimer nanoparticles with terminal lactose, or *Erythrina corralodendron* lectin can be used as agents to block AAAV infection of a cell. The synthesis of dendrimers has been described (Schchepinov, M.S., Udalova, I.A., Bridgman, A.J., Southern, E.M., 1997, Nucleic Acids Res. 25:4447-4454).

A method of inducing an immune response to AAAV in a subject comprising administering an AAAV particle comprising the capsid protein (SEQ ID NO: 11, SEQ ID NO: 12 or SEQ ID NO:13) or epitope thereof, wherein the capsid protein or epitope thereof comprises an epitope that induces an immune response in a subject. The capsid protein can also include epitopes of other (non-AAAV) proteins (as described herein) such that an immune response is directed against the non-AAAV epitope.

A method of blocking an immune response against AAAV in a subject comprising administering an AAAV particle comprising the capsid protein (SEQ ID NO: 11, SEQ ID NO: 12 or SEQ ID NO:13) or epitope thereof, wherein the capsid protein or epitope thereof comprises an epitope that blocks the immune response to AAAV in a subject. The capsid protein can also include epitopes of other (non-AAAV) proteins (as described herein) such that the immune response that is blocked is an immune response directed against the non-AAAV epitope.

A method of producing a recombinant protein is provided, comprising administering an AAAV particle comprising an exogenous nucleic acid encoding a protein to an embryonated avian egg; and b) purifying the protein from the egg. The protein purified by a method of the invention is also provided.

EXAMPLES

To understand the nature of AAAV virus and to determine its usefulness as a vector for gene transfer, it was cloned and sequenced.

Materials and Methods

Cell culture and virus propagation. 293T and COS cells were maintained in IMEM and AMEM, respectively, containing 10% FBS, DF1 cells (spontaneously immortalized chicken embryonic fibroblasts), QNR cells (quail neuroretinal cells), A549 and primary chicken embryonic fibroblasts (CEF) were maintained in DMEM supplemented with 10% FBS, primary chicken embryonic kidney cells were maintained in BME supplemented with 10% FBS, primary chicken pituitary cells were maintained in DMEM supplemented with 5% horse serum, QT6 cells (quail fibrosarcoma) were maintained in Ham's F12K supplemented with 10% FBS, LMH cells (chicken hepatoma cells) cells were maintained in Waymouth's media supplemented with 10% FBS, DT-90 (chicken lymphoblastoma cells) were maintained in DMEM supplemented with 15% FBS, 5% chicken serum and 0.015% β-mercaptoethanol. Human primary fibroblasts were obtained from Clonetics and maintained in serum-free proprietary medium supplied by the manufacturer. AAAV (ATCC, VR-865) was propagated in ten day old Spafas pathogen free embryonated chick eggs co-infected with the Phelps strain of fowl adenovirus type I (FAV1; ATCC, VR-486). AAAV at 10^4-10^7 and FAV1 at 10^5 infectious particles in saline were simultaneously injected in the chorioallantoic cavity of eggs and incubated for 96 hrs at 37 C. At the end of the incubation allantoamniotic fluids (AAFs) were harvested and clarified by centrifugation at 6000g for 10 min.

Viral DNA isolation, cloning and sequencing. Virus from infected clarified AAFs was precipitated by centrifugation at 100,000 g for 2 hr. The supernatant was discharged and the virus-containing pellet was resuspended in proteinase K digestion buffer (50 mM Tris pH=8, 20mM EDTA, 0.5% SDS, 200 μg/ml proteinase K) and

incubated at 45 C for 2 h. Following a phenol-chloroform extraction and ethanol precipitation, the viral DNA was resuspended in TE buffer containing 0.1 M Nacl. The single stranded viral DNA was annealed by heating to 95 C for 5 min followed by slow cooling to 65 C for 6 h. The annealed viral DNA was separated electrophoretically in 1% agarose gel and the double stranded AAAV DNA of approximately 4.7 kb was excised and purified using a gel extraction kit (Qiagen). The viral DNA was further processed to fill in the ends by treating with DNA polymerase (Klenow fragment) at 37 C for 15 min in the presence of dNTPs. The whole genome was then blunt end cloned in the pPCR-script cloning vector containing the LacZ gene allowing blue-white screening of ampicilin resistant colonies (Stratagene). Colonies that contained large inserts (4.7kb) were initially screened by restriction digestion and three clones were selected for sequencing. No sequence differences were found in these three clones. Sequence of the entire genome (except ITRs) was determined using an ABI 373A automated sequencer and FS dye-terminator chemistry (ABI). Due to high degree of secondary structure, ITRs were sequenced by isothermal non-cycling sequencing chemistry using radiolabeled dCTP (Epicentre). One of the clones (pAAAV) that contained the entire consensus sequence of AAAV was further used to generate packaging and vector plasmids for construction of recombinant AAAV (rAAAV) virus. The complete DNA sequence of AAAV have been submitted to GenBank (Accession number AY186198).

Sequence analysis. DNA and protein sequence alignments were performed using the Clustal W multiple sequence alignment tool of the Biology Workbench web based software (SDSC). Promoters, transcription initiation and splice sites were predicted using the Neural Network Promoter Prediction web paged software (BDGP). The presence of potential transcription binding sites was analyzed using the MatInspector computer program (54). Putative motifs in the Rep proteins were identified using the BLIMPS program that search for motifs in the Blocks protein database (28).

Southern blot hybridization. The ability of pAAAV to support self-excision,

packaging and generation of nuclease resistant wild type AAAV particles was examined. 293T cells seeded in 6-well plates were transfected using calcium phosphate co-precipitation with pAAAV alone, pAAAV plus pAd12 (a helper plasmid containing the E2 and E4 ORFs and VA RNAs of Ad5) and pAAAV plus infection with Ad5. In addition, LMH cells seeded in gelatin-coated 6-well plates were similarly transfected with pAAAV alone or with pAAAV plus infection with FAV1. After 48 hr, clarified lysates were prepared using three freeze-thaw cycles and centrifugation at 3800xg for 20 min. The lysate (~100 µl) was treated with 5 units of DNAse for 2 hr at 37 to remove vector and unpackaged progeny. Subsequently, the solution were adjusted to contain 20 mM EDTA (pH=8), 0.5 % SDS and 200 µg/ml proteinase K and incubated at 45 C for 2 hr. After one phenol-chloroform extraction, nucleic acids were precipitated with addition of an equal volume of isopropanol, and the pellet was resuspended in 30 µl of TE buffer containing 0.1 M NaCl. The samples were heated to 95 C for 5 min, slowly cooled down to 65 C at which point and incubated for 5hr. After electrophoresis and blotting, the membrane was probed with a 32P-labeled 1.2 kb BamH1 fragment of pAAAV.

Generation of recombinant AAAV particles. For production of recombinant particles we three different helper plasmids were generated and examined, pMA_3VRC, pCA_3VRC, pA_3VRC, containing the AAAV *rep* and *cap* genes under control of an MMTV, CMV or the native p5 promoters, respectively. For generation of pMA_3VRC, the *rep* and *cap* ORFs (nucleotides 243-4482) was produced by PCR with pfu polymerase (Stratagene) as specified by the manufacturer using primers containing BstZ107 and NotI sites. The PCR products were digested with BstZ107 and NotI and ligated in a BstZ107/NotI fragment of pMMTV2.1 (18) containing an MMTV promoter and SV40 polyA. For generation of pCA_3VRC, the *rep* and *cap* ORFs (nucleotides 243-4482) was produced by PCR with pfu polymerase and blunt end ligated in the pCMV- script (Stratagene) vector, which contains a CMV promoter and SV40 polyA. For generation of pA_3VRC, the *rep* and *cap* genes of AAAV including the p5 promoter and polyA signal (nucleotides 142-4516) was produced by PCR using pfu polymerase

and blunt-end ligated in pPCR-script. Orientation of inserts was verified by restriction digestion analysis, and final clones confirmed by sequencing. For generation of the vector carrying the β-galactosidase gene flanked by AAAV ITRs, the plasmid pAAAV was digested with BsmB1 (NEB). BsmB1 does not cut in the plasmid backbone but cut at positions 838, 1111, 2590, 4419 and 4530 of the AAAV genome. The resulting fragment that contained the plasmid backbone and 700 bp of AAAV genome flanked by ITRs was used to ligated a BsmB1-BsmI linker. The resulting plasmid was digested with Pml1 (cuts at nucleotide 146 of AAAV genome) and BsmI and used to ligated a BstZ107-BsmI fragment of $pAAV_2RnLacZ$ (18) that contains the β-galactosidase gene under control of an RSV promoter and SV40 polyA tail. The resulting plasmid ($pA_3VRSV\beta Gal$) was co-transfected with one of the helper plasmids described above and pAd12 in 293T cells plated in 150 cm dishes. Forty-eight hours post-transfection, cells were harvested and quantitated with a hematocytometer, and rAAAV prepared using standard CsCl gradient purification. The number of rAAAV genomes was estimated using real time quantitative PCR (QPCR) and expressed as nuclease resistant particles per cell recovered after transfections (DRP/cell). Titration of rAAAV was performed in exponentially growing CEF, DF-1, LMH, QNR, QT6, DT-90, 293T, COS and primary embryonic chicken kidney cells and non-dividing primary pituitary cells plated in 96 well plates, and transduced with serial dilutions of recombinant virus for 48 h as previously described (20).

To obtain AAAV genomic DNA for cloning, a stock of AAAV was obtained from ATCC (VR-865) and coinfected with Fowl adenovirus type I in day 10 embryonated chicken eggs. Virus was concentrated after subjecting infected allantoamniotic fluids to high-speed centrifugation. Viral DNA was released by SDS-Proteinase K digestion and purified by gel electrophoresis after annealing the complementary single strands by heating the purified DNA to 95°C and slowly cooling to 65°C. Preliminary experiments indicated that 10^5 infectious particles of FAV1 resulted in productive infection without succumbing the embryo prematurely. Co-infection with at least 10^5 infectious particles of AAAV was required to detect viral

DNA (~4.7 kb) by ethidium bromide staining. After recovery and end-filling, the double stranded AAAV genome was blunt-end ligated and cloned into pPCR-script. Several clones that contained an insert of approximately 4.7 kb were initially screened by restriction digestion and all gave bands similar in size to those previously reported (30). Three of these clones were sequenced and all gave identical sequences. One of the clones was randomly selected and used in subsequent analysis (pAAAV).

To verify that pAAAV can support self-excision, viral DNA replication, and packaging in mammalian and avian cells, viral lysates were prepared from 293T and LMH cells transfected with pAAAV and infected with wild type Ad5 or FAV1, respectively. In addition, the ability of an Ad5 plasmid to provide helper functions was examined in 293T cells. Southern blot analysis showed encapsidated (nuclease resistant particles) AAAV progeny in the presence of wtAd5 or Ad helper plasmid in 293T cells and FAV1 in LMH cells but not in the absence (Fig. 1a and b). This result suggests that pAAAV can support rescue of AAAV in mammalian and avian cells in the presence of mammalian or avian adenoviral genes.

The AAAV ITR is composed of 142 nucleotides with the first 122 forming the characteristic T-shaped palindromic structure (Fig 3), and it is 60-62% homologous with the ITRs of serotypes 2, 3, 4, and 6 and 48% homologous with AAV5. A tandem repeat of GAGY in the ITR, which serves as the binding element of Rep78 and Rep68 (RBE), is conserved between AAAV and the other AAVs (Fig. 3,4). The *trs* recognition motif of serotypes 2, 3, 4 and 6 (CCGGT'TG) is highly homologous with that of the putative AAAV *trs* (CCGGT'CG) and weekly homologous with AAV5 *trs* site (ACGGT'GT). In addition, the spacing between the RBE and the putative *trs* is similar to that found in other serotypes, a characteristic that has been shown to be essential for Rep activity (12).

It has been proposed that a potential inverted repeat flanking the core *trs* sequence of AAV serotypes might be required for Rep *trs* nicking (11). Such an

inverted repeat is not found around the AAAV *trs* sequence. This observation may indicate that Avian Rep nicking does not require any secondary structure around the core *trs* element. Methylation interference experiments have indicated the importance of the CTTTG motif found at the tip of one palindrome in AAV2 Rep binding (57). Most of this motif is conserved in AAAV ITR (CTTCG) and only one T residues is changed to C. Interestingly, the AAV4 ITR has a similar substitution in this motif (CTCTG). Thus, irrespectively of the overall nucleotide sequence homology, the secondary structure and the elements required for viral replication are conserved in the AAAV ITR.

The entire AAAV genome (Fig. 3) is 4,694 nucleotides in length and has similar organization with that of other AAVs. It has two inverted terminal repeats and two distinct ORFs. The entire genome of AAAV displays 56-65% identity at the nucleotide level with the other known AAVs. The p5 promoter region of AAAV is much shorter and shows some divergence from homologous regions of other AAV serotypes. Core regulatory elements such as the TATAA box and Ebox/USF are conserved, however YY1 and Rep binding sites are not present. This suggests that AAAV gene expression might be regulated differently from that of other AAVs. The p19 promoter, the p40 promoter, and poly(A) can also be identified in the AAAV genome by homology to those in primate AAV serotypes. Based on the general organization and sequence, these elements are highly conserved.

Clustal W protein sequence alignment indicate the left ORF of AAAV is 46-54% identical and equally divergent from that of the primate AAVs and the GPV Rep ORF (Fig. 4 a) and only 18-22% identical with the Rep ORF of other mammalian autonomous parvovirus. In comparison, the Rep ORF of isolates 1, 2, 3, 4, 6, 7 and 8 are greater than 90% similar and approximately 67-70% identical with that of AAV5 Rep ORF. The central region of the AAAV Rep ORF (aa 322 to 470), which is present in all Rep proteins, displays the greatest identity (82%) with the same region of the other AAVs and the GPV. This region of the Rep proteins is necessary for ATPase and

helicase activity and contains an ATP-binding site (aa 334 to 349) and a divalent cation binding site at amino acid residue 421 (44, 61, 65). The amino terminus (aa 1 to 251) is 42-45% similar between AAAV and the other AAVs. This region of the Rep78 and Rep68 proteins is required for DNA binding and *trs* endonuclease activities (22, 50). A tyrosine residue at 155 is homologous to the Tyr156 in AAV2 that functions as the active nucleophile in the *trs* endonuclease site (22, 62). The active site is assembled by the spatial convergence of a divalent metal ion that is tetrahedrally coordinated by Asp24, Glu83, His90 and His92. In addition Glu6 is required for the correct orientation of the two active sites imidazoles from His90 and His92 (31). All of these amino acid residues are strictly conserved among AAV serotypes including AAAV. Furthermore, a helix region important for Rep multimerization (aa 159-179) is also conserved in AAAV. The carboxyl terminal portion (aa 490-662) of the unspliced AAAV Rep proteins appears highly divergent, displaying less than 15% homology with the primate serotypes. However, a characteristic Zinc finger motif was identified using the BLIMPS algorithm. This feature is conserved in all AAV serotypes.

The right ORF of AAAV, which encodes the three viral capsid proteins, is approximately 54-57% identical to the capsid ORF of the other AAVs and the GPV (Fig. 4 b). It has been previously reported (6) that the AAAV capsid proteins VP1, VP2 and VP3 have apparent molecular weights of 92, 69 and 61 kDa, respectively, as determined by SDS-PAGE. The calculated molecular masses based on amino acid composition for VP1, VP2 and VP3 are 83, 67 and 60 kDa. We also subjected purified AAAV virions to SDS-PAGE and found that they have MW 91, 68 and 60 kDa (data not shown). As with the primate AAVs and the goose and duck autonomous parvovirus, the AAAV cap gene contains two ATG initiator codons, one for VP1 and the other for VP3. The unusual ACG initiator codon for VP2 is also conserved in AAAV.

Clustal W alignment of the VP ORFs indicated the presence of conserved and divergent regions. The N terminus of VP1 (aa 1-143), which is required for particle

formation, is relatively conserved among AAAV, AAV2, AAV4, AAV5 and GPV. However, the start site for VP2 and VP3 are found in a divergent region. Based on the published three-dimensional structure of the canine parvovirus and comparisons of parvovirus capsid sequences (15), most of the divergent regions among AAAV, AAV2, AAV4 and AAV5 and GPV are located on the exterior of the virus, thus suggesting different uptake mechanisms and altered tissue tropism.

In the present study, recombinant AAAV particles containing the gene for nuclear localized β-galactosidase were constructed. Virus was produced as previously described (19, 20) by constructing a vector plasmid containing the β-galactosidase gene under control of an RSV promoter flanked by AAAV ITRs (pA3Vβgal, Fig. 5 a), and a helper plasmid containing the AAAV rep and cap genes. Virus was isolated from 293T cell lysates by CsCl banding, and the distribution of recombinant virus across the gradient was determined by QPCR analysis of gradient fractions. The majority of packaged genomes were found in fractions with a density of 1.42 g/cm^3, which is similar to that of wt AAAV. We also examined the yield of rAAAV when using helper plasmids with the rep gene under control of three different promoters, CMV, MMTV or the native P5 promoter (Fig. 5 a). The different helper plasmids (pCA3VRC, pMA3VRC, pA3VRC) were co-transfected with pA3Vbgal and an adenovirus helper plasmid in 293T cells and rAAAV was purified from the three different CVLs using CsCl gradients. The number of rAAAV genomes was determined by QPCR. In three independent trials, the yield of rAAAV was 5-fold and 15-fold greater using the stronger CMV promoter compared with the MMTV and the native P5 promoter, respectively (Fig 5 a). This finding with rAAAV is in contrast to previous work with AAV2 that demonstrated the use of a CMV promoter inhibited the production of rAAV2 (39).

In preliminary studies, it was observed that the addition of detergents during virus purification affected infectivity. To better understand the effect of detergents, we prepared rAAAV in the presence of the following conditions: 0.5% deoxycholate, 0.5%

CHAPS, 0.5% octylglucoside (OCG) or no detergent, respectively. The virus from the four groups was purified using CsCl gradients and rAAAV genomes were quantitated using quantitative PCR. No effect was observed on yield of viral particles or density of rAAAV in the four preparations. After dialysis against PBS, transduction efficiency was measured by titration on CEF cells. Addition of OCG or CHAPS had no significant effect on transduction efficiency. However, deoxycholate which is a stronger ionic detergent reduced transduction efficiency almost 10-fold.

Tissue tropism of rAAAV was determined in CEF, DF1, LMH, DT-90, QNR, QT6, 293T, COS, primary chicken embryonic kidney cells, primary chicken pituitary cells and primary human fibroblasts and compared with that of rAAV2, rAAV4 and rAAV5 (Table 1). Table 1 shows the titers for rAAAV, rAAV2, rAAV4 and rAAV5 expressing LacZ in avian and mammalian cell lines and primary cells. Transductions were performed as described in Methods and Materials and efficiency was expressed as transducing units per 10^6 recombinant particles.

Transduction efficiency of rAAAV was 10-300 fold higher in avian cells compared with that of rAAV2, rAAV4 and rAAV5. In contrast, transduction of the mammalian cells in the panel by rAAAV was almost absent. This observation suggests that AAAV is using a different uptake or transduction mechanism compared with the primate AAVs. Interestingly, rAAAV exhibited ~15-fold higher transduction efficiency in primary chicken embryonic fibroblasts compared to immortalized embryonic fibroblasts (Fig. 5B).

The present invention also showed that AAAV ITR can function as a universal ITR for packaging with AAV2, 5 Rep proteins. Cross packaging experiments were carried out by transducing 293T cells with the two production plasmids (an ITR containing plasmid and a RepCap production plasmid) indicate and a third helper plasmid to supply adenovirus function. Forty-eight hours post transfection, cells were harvested and the amount of DNAse resistance virus measured by quantitative PCR.

Table 1

	Transducing units per 10^6 genomes			
Cell type	rAAAV	rAAV2	rAAV4	rAAV5
CEF	7140 ± 380	25 ± 3.5	84 ± 6.3	58 ± 5.7
DF-1	530 ± 35	8 ± 0.9	45 ± 4.7	60 ± 6.1
LMH	2380 ± 145	230 ± 25	34 ± 5.6	40 ± 4.9
DT-90	ND	ND	ND	ND
QNR	1260 ± 90	176 ± 18	42 ± 5.2	185 ± 26
QT6	930 ± 62	112 ± 21	23 ± 3.8	33 ± 5
Chicken Primary Embryonic Kidney cells	8080 ± 560	422 ± 46	350 ± 40	235 ± 38
Chicken Primary pituitary cells	4640 ± 375	144 ± 17	70 ± 12	91 ± 8.4
293T	ND	4500 ± 355	3130 ± 270	684 ± 57
COS	5 ± 0.7	6920 ± 420	3550 ± 165	592 ± 53
A549	ND	2190 ± 315	1360 ± 140	26 ± 4.3
Humary primary fibroblasts	ND	1990 ± 170	1130 ± 145	292 ± 31

Numbers represent the mean ± standard error from four independent transduction assays. ND=none detected.

Characterization of Binding and Transduction

The characterization of the binding and transduction requirements is important for the optimal utilization of a vector. Therefore we have examined the binding and transduction requirements of avian AAV (AAAV). To date, primate AAVs have been shown to require cell surface expression of either heparin sulfate proteoglycans (HSPG) (AAV2, 3) or sialic acid (AAV4, 5) for virus binding and attachment. However our

studies with avian AAV indicate that neither is required. Surprisingly, AAAV required a distinct form of glycosylation, terminal lactose, for efficient binding and transduction, which is unique a distinct from that of the primate AAVs.

Initial experiments with AAAV demonstrated that transduction is insensitive to competition with soluble heparin, which blocks binding with HSPG, soluble sialoconjugates, which blocks binding with sialic acid, or treatment with neuraminidase, which removes cell surface sialic acid (Figs 7, 8 and 6, respectively). Thus, Avian AAV appeared to be requiring a unique cell surface epitope. To characterize this epitope we treated DF-1 cells with several different inhibitors of glycosylation. Treatment with tunicamycin, which inhibits N-linked glycosylation, blocked both virus binding and transduction. In contrast, treatment with the O-linked inhibitor N-benzyl gal NAc had no effect (Figs 9 and 11, respectively).

Similar results were obtained with other N-linked inhibitors including N-butyl deoxynojirimycin and the unmodified form deoxynojirimycin. PDMP and Fumonisin B1, which inhibit the glycosylation of sphinogolipids and ceramides, had no effect on AAAV binding or transduction suggesting that the carbohydrate necessary for binding was attached to a protein (Fig 12). Lack of inhibition by Fumonisin B1 and PDMP and DGJ suggest lipids are not involved, but inhibition of binding and transduction with NB-DNJ and DNJ suggests glycoprotein is involved.

Initial experiments to block transduction by treatment with the protease trypsin had no effect on transduction (Fig 10). While trypsin is considered a broad specificity protease, its activity can be blocked by glycosylation; therefore we tested trypsin treatment after incubating the cells with low levels of tunicamycin which did not effectively block transduction. Treatment with trypsin or low levels of tunicamycin alone inhibited 0% or 25% of AAAV transduction respectively. However, the combination of the two inhibited greater than 90% of AAAV transduction confirming that AAAV required the presence of a N-linked glycoprotein for efficient transduction

(Fig 10).

To further identify the carbohydrate component we tested a series of lectins for the ability to block virus binding and transduction (Fig 13). These lectins are briefly described as follows:

MAA- This lectin binds glycoconjugates having galactosyl (b-1,4) N-acetylglucosamine structures. *Maackia amurensis* lectin I seems to tolerate substitution of N-acetyllactosamine with sialic acid at the 3 position of galactose;

SNA- *Sambucus nigra* lectin binds preferentially to sialic acid attached to terminal galactose in (a-2,6), and to a lesser degree, (a-2,3), linkage;

UEA-I- UEA I binds to many glycoproteins and glycolipids containing a-linked fucose residues;

PSA - This lectin has specificity toward a-linked mannose-containing oligosaccharides, with an N-acetylchitobiose-linked a-fucose residue included in the receptor sequence;

PHA-P - This lectin binds to complex carbohydrate structures on the cell surface;

MPL- This lectin prefers alpha linked N-acetylgalactosamine structures;

EEL- This lectin has a carbohydrate binding specificity toward type 1 or type 2 chain blood group B structures but will bind other oligosaccharides containing galactosyl (a-1,3) galactose;

Con A- recognizes a commonly occurring sugar structure, a-linked mannose;

BPL- Binding appears to be highest for glycoconjugates containing galactosyl (b-1,3) N-acetylgalactosamine structures but oligosaccharides with a terminal alpha linked N-acetylgalactosamine can also bind;

ERCL - *Erythrina corallodendron* has an affinity for N-acetyllactosamine, N-acetyl-D-galactosamine, lactose and D-galactose;

WGA- The receptor sugar for WGA is N-acetylglucosamine, with preferential binding to dimers and trimers of this sugar. WGA can bind oligosaccharides containing terminal N-acetylglucosamine or chitobiose, structures which are common to many serum and membrane glycoproteins; and

WGA-s succinylated wheat germ agglutinin does not bind to sialic acid residues, unlike the native form, but retains its specificity toward N-acetylglucosamine (Eur. J.

Biochem. 98, 39, 1979 and Eur. J. Biochem. 104, 147, 1980).

In agreement with the neuraminidase data, lectins MAA and SNA, which bind sialic acid, had no effect on AAAV binding or transduction (Fig 13). Furthermore, both WGA and the succinylated form, which does not bind sialic acid, both inhibited AAAV binding and transduction in agreement with the MAA and SNA data. Binding and transduction were also inhibited by *Erythrina corralodendron* lectin which binds terminal poly lactose, suggesting that AAAV may bind this carbohydrate complex. To test this hypothesis, competition experiments were carried out with soluble sialolactose conjugates or lactose complexes alone. While AAAV was inhibited by the terminal lactose conjugates, AAV5 was not, confirming the results of the lectin blocking experiments (Fig 8).

Taken together, these results indicate that AAAV requires N-linked terminal lactose present on cell surface proteins for efficient binding and entry. While other proteins may be involved in transduction, terminal lactose should be considered as a co-receptor for AAAV binding and entry. This finding was completely unexpected and very different from that of primate AAVs.

Based on this data, the use of lactose affinity chromatography (e.g., columns) for the purification of AAAV is provided. An example of lactose affinity chromatography is described by Tasumi et al., Primary structure and characteristics of a lectin from skin mucus of the Japanese eel *Anguilla japonica*, J Biol Chem. 2002 Jul 26;277(30):27305-11 (which is incorporated herein by reference).

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

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It will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

What is claimed is:

1. A nucleic acid vector comprising a pair of avian adeno-associated virus (AAAV) inverted terminal repeats and a promoter between the inverted terminal repeats.

2. The vector of claim 1, wherein the promoter is an AAV promoter p5.

3. The vector of claim 1, wherein the p5 promoter is AAAV p5 promoter.

4. The vector of claim 1, further comprising an exogenous nucleic acid functionally linked to the promoter.

5. The vector of claim 1 encapsidated in an adeno-associated virus particle.

6. The particle of claim 5, wherein the particle is an AAAV particle.

7. The particle of claim 5, wherein the particle is an AAV1 particle, an AAV2 particle, an AAV3 particle, an AAV4 particle, an AAV5, an AAV6, an AAV7, or an AAV8 particle.

8. The particle of claim 5, wherein the particle is parvovirus particle.

9. The particle of claim 5, wherein the particle is dependent parvovirus particle.

10. The particle of claim 5, wherein the particle is parvovirus particle.

11. A recombinant AAAV virion containing a vector comprising a pair of AAAV inverted terminal repeats.

12. The virion of claim 8, wherein the vector further comprises an exogenous nucleic acid inserted between the inverted terminal repeats.

13. An isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:1.

14. An isolated nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO:1.

15. An isolated nucleic acid that selectively hybridizes with the nucleic acid of claim 13.

16. An isolated nucleic acid encoding an AAAV Rep protein.

17. The nucleic acid of claim 13, wherein the avian adeno-associated virus Rep protein has the amino acid sequence set forth in SEQ ID NO:3.

18. The nucleic acid of claim 13, wherein the AAAV Rep protein has the amino acid sequence set forth in SEQ ID NO:5.

19. The nucleic acid of claim 13, wherein the AAAV Rep protein has the amino acid sequence set forth in SEQ ID NO:7.

20. The nucleic acid of claim 13, wherein the AAAV Rep protein has the amino acid sequence set forth in SEQ ID NO:9.

21. An isolated AAAV Rep protein.

22. The isolated AAAV Rep protein of claim 21, having the amino acid sequence set forth in SEQ ID NO:3, or a unique fragment thereof.

23. The isolated AAAV Rep protein of claim 21, having the amino acid sequence set forth in SEQ ID NO:5, or a unique fragment thereof.

24. An isolated antibody that specifically binds the protein of claim 18.

25. An isolated AAAV capsid protein.

26. The isolated AAAV capsid protein of claim 25 having the amino acid sequence set forth in SEQ ID NO:11.

27. The isolated AAAV capsid protein of claim 26 having the amino acid sequence set forth in SEQ ID NO:11 wherein SEQ ID NO: 11 has been modified.

28. The isolated AAAV capsid protein of claim 27, wherein the modification alters the tropism of AAAV.

29. The isolated AAAV capsid protein of claim 27, wherein the modification results in a host immune response to SEQ ID NO: 11.

30. The isolated AAAV capsid protein of claim 27, wherein the modification results in an immune response directed against AAAV.

31. An isolated antibody that specifically binds the protein of claim 25.

32. The isolated AAAV capsid protein of claim 25, having the amino acid sequence set forth in SEQ ID NO:13.

33. An isolated antibody that specifically binds the protein of claim 32.

34. The isolated AAAV capsid protein of claim 25, having the amino acid sequence set forth in SEQ ID NO:15.

35. An isolated antibody that specifically binds the protein of claim 34.

36. An isolated nucleic acid encoding the protein of claim 25.

37. The nucleic acid of claim 36, having the nucleic acid sequence set forth in SEQ ID NO:10.

38. The nucleic acid of claim 36, having the nucleic acid sequence set forth in SEQ ID NO:12.

37. The nucleic acid of claim 36, having the nucleic acid sequence set forth in SEQ ID NO:14.

38. An isolated nucleic acid that selectively hybridizes with the nucleic acid of claim 36.

39. An AAAV particle comprising a capsid protein consisting essentially of the amino acid sequence set forth in SEQ ID NO:11.

40. An isolated nucleic acid comprising an AAAV p5 promoter.

41. A method of screening a cell for infectivity by AAAV, comprising contacting the cell with AAAV and detecting the presence of AAAV in the cells.

42. A method of determining the suitability of an AAAV vector for administration

to a subject, comprising contacting an antibody-containing sample from the subject with an antigenic fragment of a protein of claim 25 and detecting an antibody-antigen reaction in the sample, the presence of a neutralizing reaction indicating the AAAV vector to be unsuitable for use in the subject.

43. A method of determining the presence in a subject of an AAAV-specific antibody comprising, contacting an antibody-containing sample from the subject with an antigenic fragment of the protein of claim 25 and detecting an antibody-antigen reaction in the sample, the presence of a reaction indicating the presence of an AAAV-specific antibody in the subject.

44. A method of delivering a nucleic acid to a cell, comprising administering to the cell an AAAV particle containing a vector comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, thereby delivering the nucleic acid to the cell.

45. The method of claim 44, wherein the AAV inverted terminal repeats are AAAV inverted terminal repeats.

46. A method of delivering a nucleic acid to a subject comprising administering to a cell from the subject an AAAV particle comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, and returning the cell to the subject, thereby delivering the nucleic acid to the subject.

47. A method of delivering a nucleic acid to a cell in a subject comprising administering to the subject an AAAV particle comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, thereby delivering the nucleic acid to a cell in the subject.

48. A method of delivering a nucleic acid to a cell in a subject having antibodies to AAV2 comprising administering to the subject an AAAV particle comprising the

nucleic acid, thereby delivering the nucleic acid to a cell in the subject.

49. An isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:20.

50. An isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO: 18.

51. A vector system for producing infectious virus particles having a characteristic of AAAV comprising: at least one vector comprising a nucleic acid selected from the group consisting of a pair of AAAV inverted terminal repeats, a nucleic acid encoding an AAAV capsid protein, and a nucleic acid encoding an AAAV rep protein.

52. The vector system of claim 51, comprising two vectors.

53. The vector system of claim 52, wherein the first vector comprises a nucleic acid encoding an AAAV Rep protein and the second vector comprises a pair of AAAV inverted terminal repeats.

54. The vector system of claim 52, wherein the first vector comprises a nucleic acid encoding an AAAV capsid protein and a nucleic acid encoding an AAAV Rep protein and the second vector comprises a pair of AAAV inverted terminal repeats.

55. The vector system of claim 52, wherein the first vector comprises a nucleic acid encoding an AAAV capsid protein and the second vector comprises a pair of AAV inverted terminal repeats.

56. The vector system of claim 55, wherein the second vector comprises a pair of AAV1 inverted terminal repeats.

57. The vector system of claim 55, wherein the second vector comprises a pair of AAV2 inverted terminal repeats.

58. The vector system of claim 55, wherein the second vector comprises a pair of AAV3 inverted terminal repeats.

59. The vector system of claim 55, wherein the second vector comprises a pair of AAV4 inverted terminal repeats.

60. The vector system of claim 55, wherein the second vector comprises a pair of AAV 5 inverted terminal repeats.

61. The vector system of claim 55, wherein the second vector comprises a pair of AAV6 inverted terminal repeats.

62. The vector system of claim 55, wherein the first vector further comprises a nucleic acid encoding an AAV1 Rep protein.

63. The vector system of claim 55, wherein the first vector further comprises a nucleic acid encoding an AAV2 Rep protein.

64. The vector system of claim 55, wherein the first vector further comprises a nucleic acid encoding an AAV3 Rep protein.

65. The vector system of claim 55, wherein the first vector further comprises a nucleic acid encoding an AAV4 Rep protein.

66. The vector system of claim 55, wherein the first vector further comprises a nucleic acid encoding an AAAV Rep protein.

67. The vector system of claim 55, wherein the first vector further comprises a nucleic acid encoding an AAV6 Rep protein.

68. The vector system of claim 51, wherein the first vector comprises a nucleic acid encoding an AAV capsid protein and the second vector comprises a pair of AAAV inverted terminal repeats.

69. The vector system of claim 68, wherein the first vector comprises a nucleic acid encoding an AAV1 capsid protein.

70. The vector system of claim 68, wherein the first vector comprises a nucleic acid encoding an AAV2 capsid protein.

71. The vector system of claim 68, wherein the first vector comprises a nucleic acid encoding an AAV3 capsid protein.

72. The vector system of claim 68, wherein the first vector comprises a nucleic acid encoding an AAV4 capsid protein.

73. The vector system of claim 68, wherein the first vector comprises a nucleic acid encoding an AAAV capsid protein.

74. The vector system of claim 68, wherein the first vector comprises a nucleic acid encoding an AAV6 capsid protein.

75. The vector system of any of claims 68 to 74, wherein the first vector further comprises a nucleic acid encoding an AAAV Rep protein.

76. The vector system of any of claims 51 to 74, wherein the second vector further comprises a promoter between the inverted terminal repeats.

77. The vector system of claim 76, wherein the promoter is functionally linked to an exogenous nucleic acid.

78. A vector comprising a pair of AAV inverted terminal repeats, a nucleic acid encoding an AAAV capsid protein and a nucleic acid encoding an AAV Rep protein.

79. The vector of claim 78, encapsidated in an dependent parvovirus particle.

80. The vector of claim 79, wherein the particle which encapsidates the vector is an AAV1 particle, an AAV2 particle, an AAV3 particle, an AAV4 particle, an AAV5 particle an AAV6 particle, and AAV7 particle, and an AAV8 particle.

81. A cell comprising an AAAV nucleic acid.

82. A population of cells comprising an AAAV nucleic acid.

83. A method of producing AAAV virus particles comprising a) transducing a cell with the nucleic acid encoding AAAV and b) isolating AAAV virus particles.

84. The virus particle produced by the method of claim 83.

85. A cell comprising the vector system of any of claims 51-77.

86. A method of producing AAAV virus particles comprising a) transducing a cell with the vector system of any of claims 51-77 and b) isolating AAAV virus particles.

87. The virus particle produced by the method of claim 86.

88. A method of inducing an immune response to AAAV in a subject comprising administering an AAAV particle comprising SEQ ID NO: 11, wherein SEQ ID NO: 11 comprises an epitope that induces an immune response in a subject.

89. A method of blocking an immune response against AAAV in a subject comprising administering an AAAV particle comprising SEQ ID NO: 11, wherein SEQ ID NO: 11 comprises an epitope that blocks the immune response to AAAV in a subject.

90. A method of producing a recombinant protein, comprising administering an AAAV particle comprising an exogenous nucleic acid encoding a protein to an embryonated avian egg; and b) purifying the protein from the egg.

91. The protein purified by the method of claim 90.

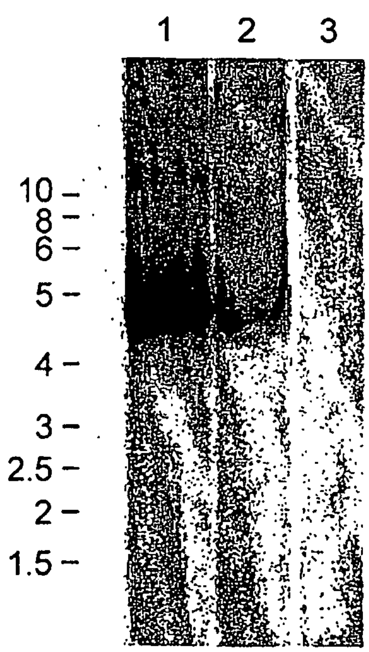

FIG.1A

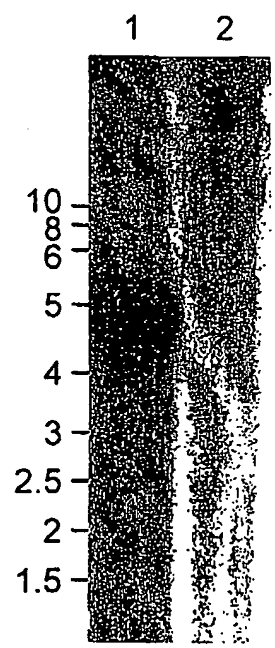

FIG.1B

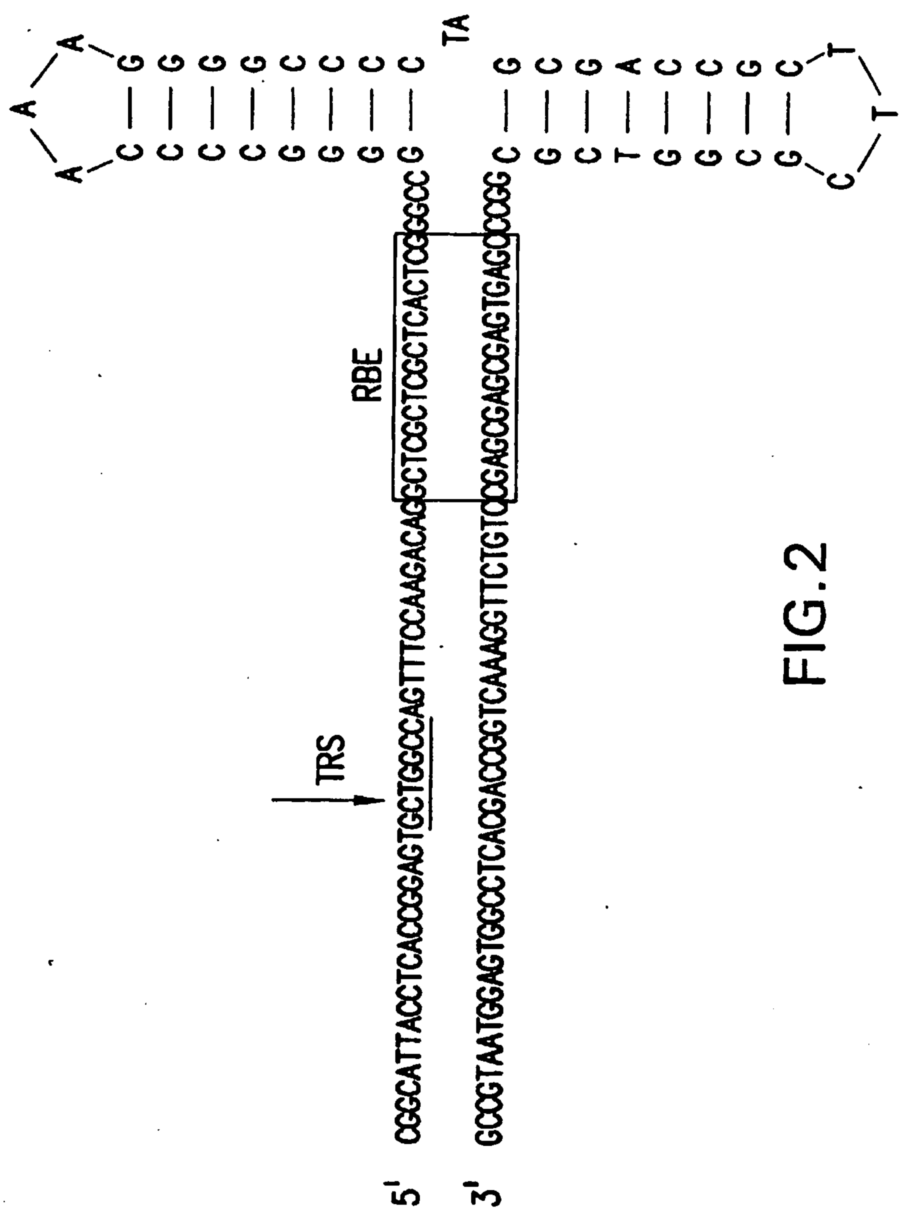

FIG.2

```
                                 RBS                                                                                                                   RBS
AAAV  -TGGC-CAGTTTC-CAAGACAGGCTCGCTCGCTCACTCG---GGCCGGGGCC--------CCAAAGGGGCCCCTAGCGACCGCTTCGCGGTCG----CGGCCCGAGTGAGCGAGCGAGCC 102
AAV2  TTGGC-CACTCCCTCTCTGCGCGCTCGCTCGCTCACTGA---GGCCGGGCGA---------CCAAAGGTCGCCCGACGCCCGGGCTTTGCCCG--GGCGGCCTCAGTGAGCGAGCGAGCG 105
AAV4  TTGGC-CACTCCCTCTATGCGCGCTCGCTCACTCACTCG---GCCCTGGAGA---------CCAAAGGTCTCCAGACTGCCGGCCTCTGGCCG--GCAGGGCCGAGTGAGTGAGCGAGCG 105
AAV5  -TGGCACTCTCCCCCCTGTCGCGTTCGCTCGCTCGCTGGCTCGTTTGGGGGGGTGGCAGCTCAAAGAGCTGCCAGACGACGGCCCTCTGGCCGTCGCCCCCCCAAACGAGCCAGCGAGCG 119
      **** *  *  * *    *  * ****** *** **      *     **          * **  *   **   *  * *     * **       *  *  ***  *******

             TRS
AAAV  TGTCTT----GGAAACTGGCCAGCACTCCG-------GT------------------------------------------------------------GAGGT-AATGC----CGTCACGTGG 150
AAV2  CGCAGA---GAGGGAGTGGCCAACTCCATCACTAGGGGTT-----------------------CCTGGAG---GGGTGG----------AGTCGTGACGTGAAT----TACGTCATAGGG 182
AAV4  CGCATA---GAGGGAGTGGCCAACTCCATCATCTAGGTTTGCCCACTGACGTCAATGTGACGTCCTAGGGTTAGGGAGGTCCCTGTATTAGCAGTCACGTGAGTGTCGTATTTCGCGGAG 222
AAV5  AGCGAACGCGACAGGGGGGAGAGTGCCACACTCTCAAGCAAG---------------GGGGTTTTGTAAGCAGTGATGTC---------ATAATGATGT-AATGCTTATTGTCACGCGA 213
       *          ** *  *                                                                                * ** * *       **

                                                                         Sp-1          E2F               TATA
AAAV  TCGGGAA-------------------------------------------TGGGAACGGGAAATCTCGCGAGAACGTAA-ACAAATATAAGACGGCGCCA-----CACGGCGCTGCGTC 220
AAV2  TTAGGGAGGTCCTGTATTAGA------------GGTCACGTGAGTGTTTTGCGACATTTTGCGACACCATGTGGTCACGCTGGGTATTTAAGCCCGAGTGAGCACGC-AGGGTCTCCATT 289
AAV4  CGTAGCGGAGCGCATACCAAGCTGCCACGTCACAGCCACGTGGTCCGTTTGCGACAGTTTGCGACACCATGTGGTCAGGA-GGGTATATAACCGCGAGTGAGCCAGCGAGGAGCTCCATT 341
AAV5  TAGTTAATGATTAACAGTCATGTGATGTGTTTTATCCA-ATAGGAAGAAAGCGCGCGTATGAGTTCTCGCGAGACTTCCG-GGGTATAAAAGACCGAGTGAACGAGCCCGCCGCCATTCT 331
                                                      *        *       *  * *         ** **    * * *     * *  *

      P5                P5
AAAV  ATAC--------GCGCGCGCGCACC-GGCGAGATGAGGTCGTACTACGAGGTCATCGTTCAGCTGCCCAACGACGTCGAGAGTCAGGTACCTGGAATCTCCGATTCGTTCGTCAACTGGA 331
AAV2  TTGAAGCGGGAGGTTTGAACGCGCA-GCCGCCATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGGGCATCTGCCCGGCATTTCTGACAGCTTTGTGAACTGGG 408
AAV4  TTGCC-CGCGAATTTTGAACGAGCA-GCAGCCATGCCGGGGTTCTACGAGATCGTGCTGAAGGTGCCCAGCGACCTGGACGAGCACCTGCCCGGCATTTCTGACTCTTTTGTGAGCTGGG 459
AAV5  TTGCTCTGGACTGCTAGAGGACCCTCGCTGCCATGGCTACCTTCTATGAAGTCATTGTTCGCGTCCCATTTGACGTGGAGGAACATCTGCCTGGAATTTCTGACAGCTTTGTGGACTGGG 451
       *            *     *  *  *  ***      *  ** **  *  *  *      * **    *** * **    **  * ** ** ** ** **     ** **   ****

AAAV  TTACGTCGCGAGAATGGACGTTGCCTGAGGACGCCGATTGGGATTTGGACCAGGTCGATCAAGTTCAACTGACGCTCGGCGACAAAATCCAACGGGAGATTCGAACTCATTGGGGGACGA 451
AAV2  TGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTCTGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGCGACTTTCTGACGGAATGGCGCCGTG 528
AAV4  TGGCCGAGAAGGAATGGGAGCTGCCGCCGGATTCTGACATGGACTTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAAAAGCTGCAACGCGAGTTCCTGGTCGAGTGGCGCCGCG 579
AAV5  TAACTGGTCAAATTTGGGAGCTGCCTCCAGAGTCAGATTTAAATTTGACTCTGGTTGAACAGCCTCAGTTGACGGTGGCTGATAGAATTCGCCGCGTGTTCCTGTACGAGTGGAACAAAT 571
      * *           *** * ****    ** * **    *  **   * * * ** **    *   ****  * *  ** *   * *  ** *    * *      * ***

AAAV  TGGCCAAAGAACCGGACTTTCACTATTTTATCCAACTGGAACAAGGTGAGGTGTTCTTTCATTTACACGTCCTGCTGGAAACGTGTTCCGTAAAGCCGATGGTACTCGGAAGATATATCC 571
AAV2  TGAGTAAGGCCCCGGAGGCCCTTTTCTTTGTGCAATTTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGGAAACCACCGGGGTGAAATCCATGGTTTTGGGACGTTTCCTGA 648
AAV4  TGAGTAAGGCCCCGGAGGCCCTCTTCTTTGTCCAGTTCGAGAAGGGGGACAGCTACTTCCACCTGCACATCCTGGTGGAGACCGTGGGCGTCAAATCCATGGTGGTGGGCCGCTACGTGA 699
AAV5  TTTCCAAG---CAGGAGTCCAAATTCTTTGTGCAGTTTGAAAAGGGATCTGAATATTTTCATCTGCACACGCTTGTGGAGACCTCCGGCATCTCTTCCATGGTCCTCGGCCGCTACGTGA 688
      *   **    * ***      *  *** * **  * **  * **       *  ** **  * ***   **  **** **        *      * *****  * **  * *   *
```

FIG.3A

```
                                                                                          Egr-1
AAAV  GACATATTCAACAAAAAATTGTGAGTAAAGTCTACTGCGCCA-CGAGCCTACGATGGAAGGATGGATGCGTGGTGACCAAGACCAAAAATTTC---GGGGGCGCGAACAAGGTCCGGGCC 687
AAV2  GTCAGATTCGCGAAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACTGGTT-CGCGGTCACAAAGACCAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTGGAT 767
AAV4  GCCAGATTAAAGAGAAGCTGGTGACCCGCATCTACCGCGGGGTCGAGCCGCAGCTTCCGAACTGGTT-CGCGGTGACCAAGACGCGTAATGGCGCCGGAGGCGGGAACAAGGTGGTGGAC 818
AAV5  GTCAGATTCGCGCCCAGCTGGTGAAAGTGGTCTTCCAGGGAATTGAACCCCAGATCAACGACTGGGT-CGCCATCACCAAGGTAAAGAAGGGC---GGAGCC---AATAAGGTGGTGGAT 801
       * ** ***       * * *      * * *  *   ** **   *    *** * **  * ** ***     **  *  ** * *  ** *****  **

                                       ETS-1               P53      TATA                            P19
AAAV  GAGTCGTATATTCCCGCCTACCTGATCCCGAAACAGCAACCGGAAGTGCAGTGGGCGTGGACTAACGTGCCCGAGTATATAAAAGCGTGCTTGCACCGAGAACTGCGTGCCAGTCTCGCG 807
AAV2  GAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCCAGTGGGCGTGGACTAATATGGAACAGTATTTAAGCGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTG 887
AAV4  GACTGCTACATCCCCAACTACCTGCTCCCCAAGACCCAGCCCGAGCTCCAGTGGGCGTGGACTAACATGGACCAGTATATAAGCGCCTGTTTGAATCTCGCGGAGCGTAAACGGCTGGTG 938
AAV5  TCTGGGTATATTCCCGCCTACCTGCTGCCGAAGGTCCAACCGGAGCTTCAGTGGGCGTGGACAAACCTGGACGAGTATAAATTGGCCGCCCTGAATCTGGAGGAGCGCAAACGGCTCGTC 921
          ** ** ***   *** ** * ** **    ** ** **  * ************* ** **    ***** *  **    ** * *     ***    * * *

                                                                                                           P19
AAAV  CGACTTCACTTCGAGGAGGCG--GGCGTCTCGCAATCCAAGGAAAATCTCGCGAGAACTGCAGACGG-CGCTCCCGTGATGCCGACCCGCGTCAGCAAACGCTACATGGAGCTCGTGGAT 924
AAV2  GCGCAGCATCTGACGCACGTGTCGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACTTCAGCCAGGTACATGGAGCTGGTCGGG 1007
AAV4  GCGCAGCATCTGACGCACGTGTCGCAGACGCAGGAGCAGAACAAGGAAAACCAGAACCCCAATTCTGACGCGCCGGTCATCAGGTCAAAAACCTCCGCCAGGTACATGGAGCTGGTCGGG 1058
AAV5  GCGCAGTTTCTGGCAGAATCCTCGCAG---CGCTCGCAGGAGGCGGCTTCGCAGCGTGAGTTCTCGGCTGACCCGGTCATCAAAAGCAAGACTTCCCAGAAATACATGGCGCTCGTCAAC 1038
        *     *     *     * * *     *              *            * *  * ** ** **                    ******* *** **

AAAV  TGGCTCGTGGAGAAGGGGATCACCACCGAGAAGGAATGGCTGCTGGAAAACAGAGAAAGCTTTCGGAGCTTTCAGGCCTCGAGCAACTCGGCGCGTCAGATCAAGACGGCCCTGCAAGGC 1044
AAV2  TGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGGAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACTCGCGGTCCCAAATCAAGGCTGCCTTGGACAAT 1127
AAV4  TGGCTGGTGGACCGCGGGATCACGTCAGAAAAGCAATGGATCCAGGAGGACCAGGCGTCCTACATCTCCTTCAACGCCGCCTCCAACTCGCGGTCACAAATCAAGGCCGCGCTGGACAAT 1178
AAV5  TGGCTCGTGGAGCACGGCATCACTTCCGAGAAGCAGTGGATCCAGGAAAATCAGGAGAGCTACCTCTCCTTCAACTCCACCGGCAACTCTCGGAGCCAGATCAAGGCCGCGCTCGACAAC 1158
      ***** *****    ** ** **  * ** *** * *** * * ***  *    *    *       ***  *  *  *  ******   *  ** ****** * **  *  *

AAAV  GCCATTCAGGAGATGCTTCTGACCAAGACGGCGGAGGACTACCTCGTCGGAAAGGATCCCGTCTCGGACGACGACATCCGTCAGAACCGCATCTACAAGATTCTGGAACTGAACCACTAC 1164
AAV2  GCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGTGGGCCAGCAGCCCGTG------GAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAACTAAACGGGTAC 1241
AAV4  GCCTCCAAAATCATGAGCCTGACAAAGACGGCTCCGGACTACCTGGTGGGCCAGAACCCGCCG------GAGGACATTTCCAGCAACCGCATCTACCGAATCCTCGAGATGAACGGGTAC 1292
AAV5  GCGACCAAAATTATGAGTCTGACAAAAAGCGCGGTGGACTACCTCGTGGGGAGCTCCGTTCCC------GAGGACATTTCAAAAAACAGAATCTGGCAAATTTTTGAGATGAATGGCTAC 1272
      **     *   ***   ***** ** *  **    ******** ** **                   ** *****      **  * ** *     **  * **  * **    ***

AAAV  GACCCAGCGTACGTGGGGAGTATTTTGGTCGGGTGGTGCCAGAAGAAATGGGGCAAGCGAAACACGCTGTGGCTGTTCGGACATGCGACCACCGGCAAGACCAACATCGCGGAGGCTATT 1284
AAV2  GATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTCGGCAAGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGCCATA 1361
AAV4  GATCCGCAGTACGCGGCCTCCGTCTTCCTGGGCTGGGCGCAAAAGAAGTTCGGGAAGAGGAACACCATCTGGCTCTTTGGGCCGGCCACGACGGGTAAAACCAACATCGCGGAAGCCATC 1412
AAV5  GACCCGGCCTACGCGGGATCCATCCTCTACGGCTGGTGTCAGCGCTCCTTCAACAAGAGGAACACCGTCTGGCTCTACGGACCCGCCACGACCGGCAAGACCAACATCGCGGAGGCCATC 1392
      ** **    ** * **     *  *   ** ***             *     *** * *****  * ***** *  ** *  ** ** ** ** ** ************* ** **
```

FIG.3B

```
AAAV GCCCATGCTGTGCCGTTCTATGGATGCGTTAACTGGACCAACGAGAACTTTCCGTTCAACGACTGCGTCGAAAAAATGATTATCTGGTGGGAGGAGGGCAAAATGACCGCCAAAGTGGTG 1404
AAV2 GCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTCAACGACTGTGTCGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTCGTG 1481
AAV4 GCCCACGCCGTGCCCTTCTACGGCTGCGTGAACTGGACCAATGAGAACTTTCCGTTCAACGATTGCGTCGACAAGATGGTGATCTGGTGGGAGGAGGGCAAGATGACGGCCAAGGTCGTA 1532
AAV5 GCCCACACTGTGCCCTTTTACGGCTGCGTGAACTGGACCAATGAAAACTTTCCCTTTAATGACTGTGTGGACAAAATGCTCATTTGGTGGGAGGAGGGAAAGATGACCAACAAGGTGGTT 1512
     ***** * ***** ** ** ** ***** *********** ** ******** ** ** ** ** ** ** ** *** * ** ************** ** *****   *** ** **

AAAV GAAACAGCCAAGGCGATTCTGGGAGGATCTCGGGTGAGAGTGGACCAAAAATGCAAAGCTTCGGTTCCGATCGAACCGACGCCGGTCATTATTACCAGTAACACCAACATGTGTTATGTC 1524
AAV2 GAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTCCTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG 1601
AAV4 GAGAGCGCCAAGGCCATCCTGGGCGGAAGCAAGGTGCGCGTGGACCAAAAGTGCAAGTCATCGGCCCAGATCGACCCAACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCGGTC 1652
AAV5 GAATCCGCCAAGGCCATCCTGGGGGGCTCAAAGGTGCGGGTCGATCAGAAATGTAAATCCTCTGTTCAAATTGATTCTACCCCTGTCATTGTAACTTCCAATACAAACATGTGTGTGGTG 1632
     **    ***** ** ** ** ** **       **** * ** ** ** ** ** **  * ** *  *  ** **  * ** ** ** **  * **   ** ** ********    **

                                                                                                          AP-1   Elk-1
AAAV ATCGACGGGAACACGACCACGTTCGAGCATAAGCAGCCGTTGGAGGACAGGATGTTTAAGCTCGAATTGCTGACTCGGTTGCCTGATGACTTTGGTAAGGTGACCAAACAGGAGGTGCGT 1644
AAV2 ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAATTTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAA 1721
AAV4 ATCGACGGAAACTCGACCACCTTCGAGCACCAACAACCACTCCAGGACCGGATGTTCAAGTTCGAGCTCACCAAGCGCCTGGAGCACGACTTTGGCAAGGTCACCAAGCAGGAAGTCAAA 1772
AAV5 GTGGATGGGAATTCCACGACCTTTGAACACCAGCAGCCGCTGGAGGACCGCATGTTCAAATTTGAACTGACTAAGCGGCTCCCGCCAGATTTTGGCAAGATTACTAAGCAGGAAGTCAAG 1752
      * ** ** **  * ** ** ** ** **  * ** **  *  * *** * ***** **  * **  *       **  *      ** ***** *** * ** ** ***** **

                                        Bcl-6       Ets-1                                        TATA
AAAV CAATTCTTCAGGTGGTCTCAGGATCACCTGACCCCTGTGATCCCAGAATTCCTAGTGCGGAAGGCGGAGTCTCGCAAAAGACCCGCCCCTTCCGGGGAAGGCT-ATATAAGCCCGACAAA 1763
AAV2 GACTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAGGGTGGAGCCAAGAAAAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGG 1841
AAV4 GACTTTTTCCGGTGGGCGTCAGATCACGTGACCGAGGTGACTCACGAGTTTTACGTCAGAAAGGGTGGAGCTAGAAAGAGGCCCGCCCCCAATGACGCAGATATAAGTGAGCCCAAGCGG 1892
AAV5 GACTTTTTTGCTTGGGCAAAGGTCAATCAGGTGCCGGTGACTCACGAGTTTAAAGTTCCCAGGGAATTGGCGGGAA------------CTAAAGGGGCGGAGA-----AATCTCTAAAAC 1855
      * ** **   *** *   *  *  *     ***  * ** **   **   * **    *   *            *    *  *  *       ****

                  P40                                             Splice
AAAV GCGGCCCGCGCTCGCAGAGCAGCAGCAGGCGTCGGAGAGCGCGGACCCGGT------TCCCACCAGGTATCGTATC--AAATGCTCGAAACATTGCGGTATGGAT-AAAATGTTGTTTCC 1874
AAV2 GTG-CGCGAGTCAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATCAACTACGCAGACAGGTACCAAAAC--AAATGTTCTCGTCACGTGGGCATGAAT-CTGATGCTGTTTCC 1957
AAV4 GCC-TGTCCGTCAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTCCGGTGGACTACGCGGACAGGTACCAAAAC--AAATGTTCTCGTCACGTGGGTATGAAT-CTGATGCTTTTTCC 2008
AAV5 GCC-CACTGG------GTGACGTCACCAATACTAGCTATAAAAGTCTGGAGAAGCGGGCCAGGCTCTCATTTGTTCCCGAGACGCCTCGCAGTTCAGACGTGACTGTTGATCCCGCTCCT 1968
     *       *     * * *        *    *  *     *  *  *    *  *   *        * ** *   **    * *
```

FIG.3C

```
AAAV  TTGCCAAATTTGTGAATCGATGAACAGAGATATTAATATTTGTGCTATTCATAAAACGACCGACTGTAAAGAGTGTTTCCCCGACTACGGGGATAAAGATGATGTAGAACTACCCCCCTG 1994
AAV2  CTGCAGACAATGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAGAGTGCTTTCCCG------------------------------------- 2040
AAV4  CTGCCGGCAATGCGAGAGAATGAATCAGAATGTGGACATTTGCTTCACGCACGGGGTCATGGACTGTGCCGAGTGCTTCCCCG------------------------------------- 2091
AAV5  CTGCGACCGCTCAATTGGAAT---TCAAGGTATGATTGCAAATGTGACTATCATGCTCA--ATTTGACAACATTTCTAACAAA------------------------------------- 2046
       ***     *     **      *         *        *    **    * * * *

AAAV  TACAGAACACAACGTGTCTCGTTGTTATCAATGTCATTCGGGCGAATTGTATCGCGTGACTTCGGACTCTGACGAGAAACCTGCCCCCGAGAGTGATGAAGGCACCGAGCCATCCTATGC 2114
AAV2  ------------------------------TGTCA-------GAATCTCAACCCGTTTCTGTCGTC-------AAAAAG------------------GCGTATC-AGAAAC--TGTGC 2093
AAV4  ------------------------------TGTCA-------GAATCTCAACCCGTGTCTGTCGTC-------AGAAAGC----------------GGACGTATC-AGAAAC--TGTGT 2147
AAV5  ------------------------------TGTGAT------GAATGTGAATATTTGAATCGGGGC-------AAAAAT-----------------GGATGTATC-TGTCACAATGTAA 2104
                                    *** *       ****  *    *   *   * *      * ***                   * * *  *  *   * *

                                                                                                             Rep78 stop
AAAV  TCCCTGCACGATTCACCACCTGATGGGCAAGAGTCACGGGTTAGTCACTTGCGCGGCGTGTCGGTTGAAAAATAGTACGTTGCATGATGACTTGGATGACGGTGATCTCGAACAATAAAT  2234
AAV2  TAC-------ATTCATCATATCATGGG-AAAGGTGCCAGAC--G---CTTGCACTGCCTGCGATCTGGTCAAT------------GTGGATTTGGATGACTGCATCTTTGAACAATAAAT  2188
AAV4  CCG-------ATTCATCACATCATGGG-GAGGGCGCCCGAG--GTGGCCTGCTCGGCCTGCGAACTGGCCAAT------------GTGGACTTGGATGACTGTGACATGGAACAATAAAT  2245
AAV5  CTC-------ACTGTCAAATTTGTCAT-GGGATTCCCCCCT--GGGAAAAGGAAAACTTGT----CAGATTTT------------GGGGATTTTGACGATGCCAATAAAGAACAGTAAAT  2198
               * *    * * *          *     *     *    * **        *            *  ** ** ** **         ***** *****

Minor splice  VP1                Splice                                       Rep68 stop
AAAV  GATTGAAATGTAGCCATGTCTCTCATTTCTGATGCGATTCCAGATTGGTTGGAGCGGTTGGTCAAAAAGGGAGTGAATGCTGCAGCTGATTTCTACCATTTGGAAAGCGGTCCTCCTCGT  2354
AAV2  GATTTAAATC-AGGTATGGCTGCCG---ATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGAAGGAATAAGAC---------AGTGGTGGAAGCTCAAACCTGGCCCACCACCA  2295
AAV4  GACTCAAACC-AGATATGACTG------ACGGTTACCTTCCAGATTGGCTAGAGGACAACCTCTCTGAAGGCGTTCGAG---------AGTGGTGGGCGCTGCAACCTGGAGCCCCTAAA  2349
AAV5  AAAGCGAGT--AGTCATGTCTTTTG---TTGATCACCCTCCAGATTGGTTGGAAGAAG---TTGGTGAAGGTCTTCGCG---------AGTTTTTGGGCCTTGAAGCGGGCCCACCGAAA  2301
       *    *   **  *** **         * *    ********** * **          *    * ** *           * * *     *  **    **  * **

AAAV  CCTAAGGCAAATCAGCAAACTCAAGAATCTCTTGAAAAGGACGATTCGAGAGGTCTCGTGTTCCCAGGCTACAATTATCTAGGCCCTTTCAACGGTCTAGATAAAGGAGAACCCGTCAAC  2474
AAV2  CCAAAGCCCGCAGAGCGGCATAA---------------GGACGACAGCAGGGGTCTTGTGCTTCCTGGGTACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGAGAGCCGGTCAAC  2400
AAV4  CCCAAGGCAAATCAACAACATCA---------------GGACAACGCTCGGGGTCTTGTGCTTCCGGGTTACAAATACCTCGGACCCGGCAACGGACTCGACAAGGGGGAACCCGTCAAC  2454
AAV5  CCAAAACCCAATCAGCAGCATCA---------------AGATCAAGCCCGTGGTCTTGTGCTGCCTGGTTATAACTATCTCGGACCCGGAAACGGTCTCGATCGAGGAGAGCCTGTCAAC  2406
       ** **  *     * *    * *                  **  *    * ***** *** * ** ** ** ** ** ** ** ** **    ***** ** **     ** ** ** ******

AAAV  GAGGCAGACGCTGCCGCCTTAGAACACG-ACAAGGCTTACGACCTCGAAATCAAGGACGGGCACAACCCGTACTTTGAGTACAACGAGGCCGACAGACGTTTCCAGGAACGTCTCAAAGA  2593
AAV2  GAGGCAGACGCCGCGGCCCTCGAGCACGTACAAAGCCTACGACCGGCAGCTCGACAGCGGAGACAACCCGTACCTCAAGTACAACCACGCCGACGCGGAGTTTCAGGAGCGCCTTAAAGA  2520
AAV4  GCAGCGGACGCGGCAGCCCTCGAGCACG-ACAAGGCCTACGACCAGCAGCTCAAGGCCGGTGACAACCCCTACCTCAAGTACAACCACGCCGACGCGGAGTTCCAGCAGCGGCTTCAGGG  2573
AAV5  AGGGCAGACGAGGTCGCGCGAGAGCACG-ACATCTCGTACAACGAGCAGCTTGAGGCGGGAGACAACCCCTACCTCAAGTACAACCACGCGGACGCCGAGTTTCAGGAGAAGCTCGCCGA  2525
        ** ****  *  **    ** **** ***    * *** **    *  *  *    **  ******* *** *  ******** * ** ***     ** *** *    **     *
```

FIG.3D

```
                                                                                                VP2
AAAV  CGATACCTCCTTTGGAGGCAATTTAGGTAAAGCCATCTTCCAGGCCAAAAAGAGGGTTCTCGAACCCTTTGGTCTGGTGGAAGACTCAAAGACGGCTCCGACCGGAGACAAGCGGAAAGG 2713
AAV2  AGATACGTCTTTTGGGGGCAACCTCGGACGAGCAGTCTTCCAGGCGAAAAAGAGGGTTCTTGAACCTCTGGGCCTGGTTGAGGAACCTGTTAAGACGGCTCCGGGAAAAAAGAGGCCGGT 2640
AAV4  CGACACATCGTTTGGGGGCAACCTCGGCAGAGCAGTCTTCCAGGCCAAAAAGAGGGTTCTTGAACCTCTTGGTCTGGTTGAGCAAGCGGGTGAGACGGCTCCTGGAAAGAAGAGACCGTT 2693
AAV5  CGACACATCCTTCGGGGGAAACCTCGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCGAACCTTTTGGCCTGGTTGAAGAGGGTGCTAAGACGGCCCCTACCGGAAAGCGGATAGA 2645
       ** ** ** ** ** ** **  * **   **  **** ***** ** ** ******** *****  * ** ***** **  *          * *  *  *      *** *

AAAV  CGAAGACGAACCTCGTTTGCCCGACACTTCTTCACAGACTCCCAAGAAAAACAAGAAGCCTCGCAAGGAAAGACCTTCC--------GGCGGGGCAGAAGATCCGGGCGAAGGCACCTCT 2825
AAV2  AGAGCACTCTCCTGTGGAGCCAGACTCCTCCTCGGGAACCGGAAAGGCGGGCCAGCAGCCTGCAAGAAAAAGATTGAATTTTGGTCAGACTGGAGACGCAGACTCAGTACCTGACCCCCA 2760
AAV4  GATTGAATCCCCCCAGCAGCCCGACTCCTCCACGGGTATCGGCAAAAAAGGCAAGCAGCCGGCTAAAAAGAAGCTCGTTTTCGA--AGAC--GAAAC-TGGAGCAGGCGACGGACCCCCT 2808
AAV5  CGACCACTTTCC-AAAAAGAAAGAAGGCTC-----GGACCGAAGAGGACTCCAAGCCTTCCAC----------CTCGTC-------AGACGCCGAAGCTGGACCCAGC----GGATCCCA 2738
         *   **    *  **   **     *     *     * **    *                            * *    *          *    .*   * *

                                         VP3
AAAV  TCCAACGCTGGAG-CAGCAGCACCCGCCTCTAGTGTGGGATCATCTATCATGGCTGAAGGAGGTGGCGGCCCAGTGGGCGATGCAGGCCAGGGTGCCGATGGAGTGGGCAATTCCTCCGG 2944
AAV2  GCCTCTCGGACAGCCACCAGCAGCCCCCTCTGGTCTGGGAACTAATACGATGGCTACAGGCAGTGGCGCACCAATGGCAGACAATAACGAGGGCGCCGACGGAGTGGGTAATTCCTCCGG 2880
AAV4  G----AGGGATCAACTTCCGGAGCCATGTCTGAT------GACAGTGAGATGCGTGCAGCAGCTGGCGGAGCTGCAGTCGAGGGCGGACAAGGTGCCGATGGAGTGGGTAATGCCTCGGG 2918
AAV5  GCAGCTGCAAATCCCAGCCCAACCAGCCTCAAGTTTGGGAGCTGATACAATGTCTGCGGGAGGTGGCGGCCCATTGGGCGACAATAACCAAGGTGCCGATGGAGTGGGCAATGCCTCGGG 2858
                 *  *  * *   **  *         *  ***  *  *   *****   *    *  **       * ** ***** ******** *** **** **

AAAV  AAATTGGCATTGCGATTCCCAATGGCTGGAAAACGGAGTCGTCACTCGAACCACCCGAACCTGGGTCTTGCCCAGCTACAACAACCACCTGTACAAACGAATCCAAGGACCCAGCGGAGG 3064
AAV2  AAATTGGCATTGCGATTCCACATGGATGGGCGACAGAGTCATCACCACCAGCACCCGAACCTGGGCCCTGCCCACCTACAACAACCACCTCTACAAACAAATTTCCAGCCAATCAGGAGC 3000
AAV4  TGATTGGCATTGCGATTCCACCTGGTCTGAGGGCCACGTCACGACCACCAGCACCAGAACCTGGGTCTTGCCCACCTACAACAACCACCTCTACAAGCGA-CTCGGAGAGAGCCTGCAGT 3037
AAV5  AGATTGGCATTGCGATTCCACGTGGATGGGGGACAGAGTCGTCACCAAGTCCACCCGAACCTGGGTGCTGCCCAGCTACAACAACCACCAGTACCGAGAGATCAAAAGCGGCTCCGTCGA 2978
        ****************   ***   *   *  ***  **     **** *********   ****** *************  ***                *     * *

AAAV  CGACA---ACAACAACAAATTCTTTGGATTCAGCACCCCCTGGGGATACTTTGACTACAATCGATTCCACTGCCACTTTTCCCCGCGAGACTGGCAACGACTCATCAACAACAACTGGGG 3181
AAV2  CTCGA---ACGACAATCACTACTTTGGCTACAGCACCCCTTGGGGGTATTTTGACTTCAACAGATTCCACTGCCACTTTTCACCACGTGACTGGCAAAGACTCATCAACAACAACTGGGG 3117
AAV4  C-----------CAACACCTACAACGGATTCTCCACCCCCTGGGGATACTTTGACTTCAACCGCTTCCACTGCCACTTCTCACCACGTGACTGGCAGCGACTCATCAACAACAACTGGGG 3146
AAV5  CGGAAGCAACGCCAACGCCTACTTTGGATACAGCACCCCCTGGGGGTACTTTGACTTTAACCGCTTCCACAGCCACTGGAGCCCCCGAGACTGGCAAAGACTCATCAACAACTACTGGGG 3098
      *            ***   * *   ** * *  ****** ***** ** *******  **  * ****** ******      ** ** ********  ************* *******
```

FIG.3E

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```
AAAV CATCCGTCCCAAAGCGATGCGCTTTAGACTCTTTAACATCCAGGTTAAAGAGGTCACGGTCCAAGACTTCAACACCACCATCGGCAACAACCTCACCAGTACGGTCCAGGTCTTTGCGGA 3301
AAV2 ATTCCGACCCAAGAGACTCAACTTCAAGCTCTTTAACATTCAAGTCAAAGAGGTCACGCAGAATGACGGTACGACGACGATTGCCAATAACCTTACCAGCACGGTTCAGGTGTTTACTGA 3237
AAV4 CATGCGACCCAAAGCCATGCGGGTCAAAATCTTCAACATCCAGGTCAAGGAGGTCACGACGTCGAACGGCGAGACAACGGTGGCTAATAACCTTACCAGCACGGTTCAGATCTTTGCGGA 3266
AAV5 CTTCAGACCCCGGTCCCTCAGAGTCAAAATCTTCAACATTCAAGTCAAAGAGGTCACGGTGCAGGACTCCACCACCACCATCGCCAACAACCTCACCTCCACCGTCCAAGTGTTTACGGA 3218
      *  * ***        *      * *    **** ***** ** ** ** *********        **       ** **  * *  ** ***** ***    ** ** **  * *** * **

AAAV CAAGGACTACCAACTGCCGTACGTCCTCGGATCGGCTACCGAAGGCACCTTCCCGCCGTTCCCAGCGGATATCTACACGATCCCGCAGTACGGGTACTGCACGCTAAACTACAACAACG- 3420
AAV2 CTCGGAGTACCAGCTCCCGTACGTCCTCGGCTCGGCGCATCAAGGATGCCTCCCGCCGTTCCCAGCAGACGTCTTCATGGTGCCACAGTATGGATACCTCACCCTGAACAACGGGAGT-- 3355
AAV4 CTCGTCGTACGAACTGCCGTACGTGATGGATGCGGGTCAAGAGGGCAGCCTGCCTCCTTTTCCCAACGACGTCTTTATGGTGCCCCAGTACGGCTACTGTGGACTGGTGACCGGCAACAC 3386
AAV5 CGACGACTACCAGCTGCCCTACGTCGTCGGCAACGGGACCGAGGGATGCCTGCCGGCCTTCCCTCCGCAGGTCTTTACGCTGCCGCAGTACGGTTACGCGACGCTGAACCGCGACAAC-- 3336
     *      *** * ** ** *****  * *       *       * **    * * **  * ** **      *  ***  * * * ** ***** ** ***       **      *   *

AAAV -AGGCGGTGGATCGTTCG-------GCCTTCTACTGTCTGGACTACTTTCCCTCAGACATGCTGCGGACAGGAAATAACTTTGAGTTTACTTACACCTTCGAGGACGTTCCTTTCCATAG 3532
AAV2 CAGGCAGTAGGACGCTC-------TTCATTTTACTGCCTGGAGTACTTTCCTTCTCAGATGCTGCGTACCGGAAACAACTTTACCTTCAGCTACACTTTTGAGGACGTTCCTTTCCACAG 3468
AAV4 TTCGCAGCAACAGACTGACAGAAATGCCTTCTACTGCCTGGAGTACTTTCCTTCGCAGATGCTGCGGACTGGCAACAACTTTGAAATTACGTACAGTTTTGAGAAGGTGCCTTTCCACTC 3506
AAV5 -ACAGAAAATCCCACCGAGAGGAGCAGCTTCTTCTGCCTAGAGTACTTTCCCAGCAAGATGCTGAGAACGGGCAACAACTTTGAGTTTACCTACAACTTTGAGGAGGTGCCCTTCCACTC 3455
                                   ** * *** ** ** ********       * ****** * ** ** ** ******     * *   ****   ** *** * ** ** *****

AAAV CATGTTTGCCCACAACCAGACGCTAGACCGGCTGATGAATCCCCTCGTGGATCAGTACCTCTGGGCTTTCAGCTCCGTCAGCCAA-----------GCAGGCTCATCTGGACGAGCTCTT 3641
AAV2 CAGCTACGCTCACAGCCAGAGTCTGGACCGTCTCATGAATCCTCTCATCGACCAGTACCTGTATTACTTGAGCAGAACAAACACT----CCAAGTGGAACCACCACGCAGTCAAGGCTTC 3584
AAV4 GATGTACGCGCACAGCCAGAGCCTGGACCGGCTGATGAACCCTCTCATCGACCAGTACCTGTGGGGACTGCAATCGACCACCACCGGAACCACCCTGAATGCCGGGACTGCCA--CCACC 3624
AAV5 CAGCTTCGCTCCCAGTCAGAACCTGTTCAAGCTGGCCAACCCGCTGGTGGACCAGTACTTGTACCGCTTCGTGAGCACAAATA--------------ACACTGGCGGAGTC-------C 3553
      *  *  ** * **  ****  **   *   **    ** ** **  * ** ****** * *       *         *                    *          * *

AAAV CATTACTCGCGGGCGACTAAAACCAACATGGCGGCTCAATATAGGAACTGGTTACCTGGGCCTTTCTTCCGTGATCAGCAAATCTTTACGGGCGCTAGCAACATCACTAAAAATAACGTC 3761
AAV2 AGTTTTCTCAGGCCGGAGCG-AGTGACATTCGGGACCAGTCTAGGAACTGGCTTCCTGGACCCTGTTACCGCCAGCAGCGAGTATCAAAGACATCTGCGGATAACAACAACAGTGAATAC 3703
AAV4 AACTTTACCAAGCTGCGGCCTACCAACTTTTCCAACTTTAAAAAGAACTGGCTGCCCGGGCCTTCAATCAAGCAGCAGGGCTTCTCAAAGACTGCCAATCAAAACTACAA-GATCCCTGC 3743
AAV5 AGTTCAACAAGAACCTGGCCGGGAGATACGCC-AACACCTACAAAAACTGGTTCCCGGGGCCCATGGGCCGAACCCAGGGCTGGAACCTGGGCTCCGGGGTCAACCGCGCCAGTGTCAGC 3672
       *                  *                 *  ****** * ** ** **        *      ***            *    *      * *         *    *

AAAV TTTAGCGTTTGGGAAAAAGGCAAGCAATGGGAACTCGACAATCGGACCAACCTAATGCAGCCCGGTCCTGCGGCAGCGACCACCTTTAGCGGAGAACCTGACCGTCAAG-------CCAT 3874
AAV2 TCGTGGACT--GGAG----CTACCAAGTACCACCTCAATGGCA------GAGACTCTCTGGTGAATCCGGCCATGGCAAGCCACAAGGACGATGAAGAAAAGTTTTTTC------CTCAG 3805
AAV4 CACCGGGTC--AGACAGTCTCATCAAATACGAGACGCACAGCACTC-TGGACGGAAGATGGAGTGCCCTGACCCCCGGACCTCCAATGGCCACGGCTGGACCTGCGGACAGCAAGTTCAG 3860
AAV5 GCCTTCGCC--ACGA----CCAATAGGATGGAGCTCGAGGGCGCGAGTTACCAGGTGCCCCCGCAGCCGAACGGCATGACCAACAACCTCCAGGGCAGCAACACCTATG----CCCTGGA 3782
                     *       *    *                          **       * *  *     *  *
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FIG.3F

```
AAAV GCAAAACACGCTGGCTTTTAGCAGGACCGTCTACGATCAAACGACCGCCACGACCGATCGTAACCAGATACTCATCACCAACGAAGACGAAATCAGACCCACCAACTCGGTCGGTATCGA 3994
AAV2 -AGCGGGGTTCTCATCTTTGGGAAGCA---AGGCTCAGAGAAAACAAATGTGAACATTGAAAAGGTCATGATT---ACAGACGAAGAGGAAATCGGAACAACCAATCCCGTGGCTACGGA 3918
AAV4 CAACAGCCAGCTCATCTTTGCGGGGCCT--AAACAGAACGGCAACACG-GCCACCGTACCCGGGACTCTGATCTTCACCTCTGAGGAGGAGCTGGCAGCCACCAACGCCACCGATACGGA 3977
AAV5 GAACACTATGATCTTCAACAGCCAGCCGGCGAACCCGGGCACCACCGCCACGTACCTCGAGGGCAACATGCTCATCACCAGCGAGAGCGAGACGCAGCCGGTGAACCGCGTGGCGTACAA 3902
               *         *       *       **        *            * *   **   **   **      *   **       *     *

AAAV CGCGTGGGGAGCAGTTCCCACCAACAACCAGTCGATCGTGACCCCCGGCACTCGCGCGGCCGTCAACAATCAAGGGGCGCTTCCCGGGATGGTGTGGCAAAACAGAGACATTTACCCTAC 4114
AAV2 GCAGTATGGTTCTGTATCTACCAACCTCCAGAGAGGCAACAGACAAGCAGCTACCGCAGATGTCAACACACAAGGCGTTCTTCCAGGCATGGTCTGGCAGGACAGAGATGTGTACCTT-C 4037
AAV4 CATGTGGGGCAACCTACCTGGCGGTGACCAGAGCAACAGCAACCTGCCGACCGTGGACAGACTGACAGCCTTGGGAGCCGTGCCTGGAATGGTCTGGCAAAACAGAGACATTTACTAC-C 4096
AAV5 CGTCGGCGGGCAGATGGCCACCAACAACCAGAGCTCCACCACTGCCCCCGCGACCGGCACGTACAACCTCCAGGAAATCGTGCCCGGCAGCGTGTGGATGGAGAGGGACGTGTACCTC-C 4021
          **    *  *  *    ****    *  *       *    *       *       *    * ** ** *  ** ***   * ** **  * ***   *

AAAV AGGGACCCATTTGG-CCAAAATTCCCGACACTGACAATCACTTCCATCCGTCCCCGCTTATTGGGCGGTTTGGCTGCAAGCATCCCCCTCCCCAGATTTTCATTAAAAACACACCCGTCC 4233
AAV2 AGGGGCCCATCTGGGCAAAGATTCCACACACGGACGGACATTTTCACCCCTCTCCCCTCATGGGTGGATTCGGACTTAAACACCCTCCTCCACAGATTCTCATCAAGAACACCCCGGTAC 4157
AAV4 AGGGTCCCATTTGGGCCAAGATTCCTCATACCGATGGACACTTTCACCCCTCACCGCTGATTGGTGGGTTTGGGCTGAAACACCCGCCTCCTCAAATTTTTATCAAGAACACCCCGGTAC 4216
AAV5 AAGGACCCATCTGGGCCAAGATCCCAGAGACGGGGGCGCACTTTCACCCCTCTCCGGCCATGGGCGGATTCGGACTCAAACACCCACCGCCCATGATGCTCATCAAGAACACGCCTGTGC 4141
     * ** ***** *** * ** ** **  * ** *    ** ** ** ** ** **    ** **  * ** **    ** ** ** ** **    **  * ** ** ***** ** ** *

AAAV CTGCCAACCCTTCGGAAACGTTCCAGACGGCCAAAGTGGCCTCCTTCATCAACCAGTACTCGACCGGACA-GTGCACCGTCGAAATCTTTTGGGAACTCAAGAAGGAAACCTCCAAGCGC 4352
AAV2 CTGCGAATCCTTCGACCACCTTCAGTGCGGCAAAGTTTGCTTCCTTCATCACACAGTACTCCACGGGACACGGTCAGCGTGGAGATCGAGTGGGAGCTGCAGAAGGAAAACAGCAAACGC 4277
AAV4 CTGCGAATCCTGCAACGACCTTCAGCTCTACTCCGGTAAACTCCTTCATTACTCAGTACAGCACTGGCCA-GGTGTCGGTGCAGATTGACTGGGAGATCCAGAAGGAGCGGTCCAAACGC 4335
AAV5 CCGGAAATATC---ACCAGCTTCTCGGACGTGCCCGTCAGCAGCTTCATCACCCAGTACAGCACCGGGCA-GGTCACCGTGGAGATGGAGTGGGAGCTCAAGAAGGAAAACTCCAAGAGG 4257
     * *  **         *  ***          *       ****** *  ******  ** ** ** *      **  * **    ***** *  *******      *** *

AAAV TGGAACCCCGAAATCCAGTTCACCTCCAACTTT----GGCAACGCGGCCGA-CATCCAGTTTGCCGTCTCCGACACGGGATCCTATTCCGAACCTCGTCCCATCGGTACCCGTTACCTTA 4467
AAV2 TGGAATCCCGAAATTCAGTACACTTCCAACTAC----AACAAGTCTGTTAATCGTGGACTTA-CCGTGGATACTAATGGCGTGTATTCAGAGCCTCGCCCCATTGGCACCAGATACCTGA 4392
AAV4 TGGAACCCCGAGGTCCAGTTTACCTCCAACTACGGACAGCAAAACTCTCTGTTGTGGGCTCC-C----GATGCGGCTGGGAAATACACTGAGCCTAGGGCTATCGGTACCCGCTACCTCA 4450
AAV5 TGGAACCCAGAGATCCAGTACACAAACAACTAC----AACGA-CCCCCAGTTTGTGGACTTTGCCCCGGACAGCACCGGGGAATACAGAACCACCAGACCTATCGGAACCCGATACCTTA 4372
     ***** ** **  * ****  **   *****        * * *        *    *   *           **   **      *  *  * ** ** *** * ***** *

            VP-stop                          Poly-A
AAAV CCAAACCTCTGTAA------ATTAA-----------------ACCCTTCAA-TAAACCG---TTTATGCGTAACTGTATTTCCGTCTC----CTGTCGTTATTCAGTCACATGA------ 4550
AAV2 CTCGTAATCTGTAATTGCTTGTTAATCAATAAACCGTTTAATTCGTTTCAGTTGAACTTTGGTCTCTGCGTATTTCTT-TCTTATCTAGTTTCCATGGCTAC---GT-AGATAAGTAGC- 4506
AAV4 CCCACCACCTGTAATAACCTGTTAATCAATAAACCGGTTTATTCGTTTCAGTTGAACTTTGGTCTCCGTGTCCTTCTTATCTTATCTCGTTTCCATGGCTACTGCGT-ACATAAGCAGCG 4569
AAV5 CCCGACCCCTTTAA---CCCATTCAT---------GTCGCATACCCTCAA--TAAACCGTG-TATTCGTGTCAGTAAAATACTGCCTC----TTGTGGTCATTCAAT-GAATAA-CAGC- 4470
     *       ** ***      ** *                * *  * * ***    * *  * **   *    *     **      * * *     *  ** *
```

FIG.3G

```
AAAV  --------------------------------TGCGG------CATTACCTC-------ACCGGAGTGCTGG-------CCAGTTTCCAAGACAGGCTCGCTCGCTCACTCGGGCCGG-G 4617
AAV2  ------------------------------ATGGCGGGTTAATCATTAACTACAAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGG-- 4594
AAV4  GCCTGCGGCGCTTGCGCTTCGCGGTTTACAACTGCCGGTTAATCAGTAACTTCTGGCAAACCTAGATGATGGAGTTGGCCACATTAGCTATGCGCGCTCGCTCACTCACTCGGCCCTG-- 4686
AAV5  -------------------------------TTACAACATCTACAAAACCCCCTTGCTTGAGAGTGTGGCACTCTCCCCCCTGTCGCGTTCGCTCGCTCGCTGGCTCGTTTGGGGGGGCG 4559
                                     *        ** .* *             **            *             * *******  ***  *  *    *

AAAV  GCCCCAAAGGGGCCCCTAGCGACCG---CTTCGCGGTCGCGGCCCG-----------AGTGAGCGAGCGAGCCTGTCTTG-GAAACTG-GCCA- 4694
AAV2  GCGACCAAAGGTCGCCCGACGCCCGGG-CTTTGCCCGGGCGGCCTC-----------AGTGAGCGAGCGAGCGCGCAGAGAGGGAGTG-GCCAA 4675
AAV4  GAGACCAAAGGTCTCCAGACTGCCGGC-CTCTGGCCGGCAGGGCCG-----------AGTGAGTGAGCGAGCGCGCATAGAGGGAGTG-GCCAA 4767
AAV5  ACGGCCAGAGGGCCGTCGTCTGGCAGCTCTTTGAGCTGCCACCCCCCCAAACGAGCCAGCGAGCGAGCGAACGCGACAGGGGGGAGAGTGCCA- 4652
          * *  ** *     *   *   ** *        *            ** *** ****** *  *    * *  *  * ****
```

FIG.3H

```
        P5
AAAV  ----------MRSYYEVIVQLPNDVESQVPGISDSFVNWITSREWTLPEDADWDLDQVDQVQLTLGDKIQREIRTHWGTMAKEPDFHYFIQLEQGEVFFHLHVLLETCSVKPMVLGRYIR 110
AAV2  ----------.PGF..IVIKV.S.LDGHL..........VAEK..E..P.S.M..NLIE.AP..VAE.L..DFL.E.RRVS.A.EALF.V.F.K..SY..M...V..TG..S.....FLS 110
AAV4  ----------.PGF..IVLKV.S.LDEHL........S.VAEK..E..P.S.M..NLIE.AP..VAE.L...FLVE.RRVS.A.EALF.V.F.K.DSY....I.V..VG..S..V...VS 110
AAV5  ----------.ATF.....RV.F...EHL........D.V.GQI.E..PES.LN.TL.E.P...VA.R.R.VFLYE.NKFS.Q-ESKF.V.F.K.SEY....T.V..SGISS......VS 109
GP    MALSRPLQISSDKF....IR.SS.IDQD...L.LN..E.LSTGV.EPTG--I.NMEH.NLPMV..AE..KNIFIQR.NQFNQD-ETDF.F...E.SEYI...CCIAQGN.RSF.....MS 117

                                        Dimerization domain                                    P19
AAAV  HIQQKIVSKVYCATSLRWKDGCVVTKTKN-FGGANKVRAESYIPAYLIPKQQPEVQWAWTNVPEYIKACLHRELRASLARLHFEEAGVSQSKENLARTADG-APVMPTRVSKRYMELVDW 228
AAV2  Q.RE.LIQRI.RGIEPTLPNWFA....R.GA..G...VD.C...N..L..T...L......MEQ.LS...NLTE.KR.VAQ.LTHVSQT.EQNKENQNPNSD...IRSKT.A......G. 230
AAV4  Q.KE.L.TRI.RGVEPQLPNWFA....R.GA..G...VDDC...N..L..T...L......MDQ..S...NLAE.KR.VAQ.LTHVSQT.EQNKENQNPNSD...IRSKT.A......G. 230
AAV5  Q.RAQL.KV.FQGIEPQIN.WVAI..V.K--......VDSG......L..V...L......LD..KL.A.NL.E.KR.VAQFLA.SSQRS-Q.AASQREFSAD..IKSKT.QK..A..N. 226
GP    Q.KDS.IRD..EGKQIKIP.WFAI....R--..Q..TVTAA..LH.....K...L...F..M.LFTA.A.CLQK.QE.LDAFQ.SDLAAP---LPDPQ.STV..LISN.AA.N.SN.... 232

                                                                                                              ATP binding
AAAV  LVEKGITTEKEWLLENRESFRSFQASSNSARQIKTALQGAIQEMLLTKTAEDYLVGKDPVSDDDIRQNRIYKILELNHYDPAYVGSILVGWCQKKWGKRNTLWLFGHATTGKTNIAEAIA 348
AAV2  ..D....S..Q.IQ.DQA.YI..N.A...RS...A..DN.GKI.S.....P.....QQ..--E..SS...........G...Q.AA.VFL..AT..F.....I....P............ 348
AAV4  ..DR...S..Q.IQ.DQA.YI..N.A...RS...A..DN.SKI.S.....P.....QN.P--E..SS.....R....M.G...Q.AA.VFL..A...F.....I....P............ 348
AAV5  ...H...S..Q.IQ..Q..YL..NSTG..RS...A..DN.TKI.S....S.V.....SSVP--E..SK...WQ.F.M.G.....A....Y....RSFN....V..Y.P............ 344
GP    .I.M...S..Q..T.....Y.....T.SNN..V.A..EN.RA.........T...I.....--L..TK..V.Q..KM.N.N.Q.I....C..VKREFN...AI..Y.P............ 350

AAAV  HAVPFYGCVNWTNENFPFNDCVEKMIIWWEEGKMTAKVVETAKAILGGSRVRVDQKCKASVPIEPTPVIITSNTNMCYVIDGNTTTFEHKQPLEDRMFKLELLTRLPDDFGKVTKQEVRQ 468
AAV2  .T...................D..V..............S........K........S.AQ.D.....V.......A.....S.....Q...Q......F..TR...DH..........KD 468
AAV4  .....................D..V..............S........K........S.AQ.D.....V.......A.....S.....Q...Q......F..TK...EH..........KD 468
AAV5  .T...................D..L..........N...S........K........S..Q.DS....V.......V.V...S.....Q..........F..TK...P.....I.....KD 464
GP    .....................D..L..........N...S........A........G..C.............D..MIV...S..M..RI...E...QIV.SHK.EPS...IS.K...E 470
                              NLS                  NLS                               Zing finger domain

AAAV  FFRWSQDHLTPVIPEFLVRKAESRKRP-----------APSGEGYISPTKRPALAEQQQASESADPVP----------TRYRIKCSKHCGMDKMLFPCQICESMNRDINICAIHKTTDCK 567
AAV2  ....AK..VVE.EH..Y.K.GGAK...-----------...DADISE.KRVRESVA.PST.DAEASIN--------YAD..QN...R.V..NL.....RQ..R..QNS...FT.GQK..L 569
AAV4  ....AS..V.E.TH..Y...GGA....------------..NDADISE.KRACPSVA.PST.DAEA..D--------YAD..QN...R.V..NL.....RQ..R..QNVD..FT.GVM..A 569
AAV5  ..A.AKVNQV..TH..K.PRELAGTKGAEKSLKRPLGDVTNTSYKSLEKRARLSFVPETPRS.DVT.DPAPLRPLNWNS..DC..DY.AQF.NISNK.DE..YL..GK.G.IC.NV.H.Q 584
GP    ..K.AN.N.V..VS..K..TN.QTNLP-----------E.VP.RANE.EEP.KIWAPPTRE.LEELLR-------ASPELFSSVAPIPVTPQNSPE.KRSRNNYQVRCALHTYDNSM.VF 571
                  Zing finger domain                         Zing finger domain

AAAV  ECFPDYGDKDDVELPPCTEHNVSRCYQCHSGELYRVTSDSDEKPAPESDEGTEPSYAPCTIHHLMGKSHGLVTCAACRLKNSTLHDDLDDGDLEQ 662
AAV2  ....VS----------------------------V.E.Q----.VSVVKK-AYQKL.Y...I...VP-D-A.T..D.V.----V....CIF.. 621
AAV4  ....VS----------------------------V.E.Q----.VSVVRKRTYQKL.P...I..RAP-E.A.S..E.A.----V....CDM.. 623
AAV5  I.HGIP----------------------------IPPWE----K.--------------------------NLSDFG------.F..ANK.. 610
GP    ..MECEKAN---------------------------------FP.FQPLG.N.CDEHGWYDCAICKELKNEL.EI----EHVFE....AEN.. 627
```

FIG.4A

```
AAAV  MSLISDAIPDWLERLVKKGVNAAADFYHLESGPPRPKANQQTQ------ESLEKDDSRGLVFPGYNYLGPFNGLDKGEPVNEADAAALEHDKAYDLEIKDGHNPYFEYNEADRRFQERLK  114
AAV2  .A-ADGYL.....---DTLSEGIRQWWK.KP...P..PAERHK-----------.......L..K.............................RQLDS.D...LK..H..AE......  105
AAV4  .T--DGYL.....---DNLSEGVREWWA.QP.A.K......H.-----------.NA....L..K....G..........A.............QQL.A.D...LK..H..AE..Q..Q  104
AAV5  ..-FV.HP.....----EVGEGLRE.LG..A...K..P...H.-----------.QA....L........G....R.....R..EV.R....IS.NEQLEA.D...LK..H..AE...K.A  104
GP    ..TFL.SFEE.Y.-------T...SWRN.KA.A.Q..P...S.SVSPDR.PER..NN..F.L...K....G.......P...K..SV.........QQL.A.D...IKF.H..QD.IDS.Q  113

                                              VP2                                                                        VP3
AAAV  DDTSFGGNLGKAIFQAKKRVLEPFGLVEDS-KTAPTGDKRKGEDEPRLPDTSSQTPKKNKKPRKERPSGGAEDPGEGTSSNAGAAAPASSVG--SSIMAEGGGGPVGDAGQGADGVGNSS  231
AAV2  E.........R.V..........L....EPV....GKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTG.ADSVPDPQPLGQ...APSGLGTNT..T.S.A.MA.NNE.........  225
AAV4  G.........R.V..........L....QAGE...GKKRPLI.SPQQPDSSTGIGKKGKQPAKKKLVFEDETGAGD.PPEGSTSGAM.DDS-----E.RAAA..AAVEG.........A.  219
AAV5  ............V...............EGA......KRIDDHFPK.KKARTEEDS.PSTSSDA.AGPSGSQQLQIPAQPASSLGADTM.A---------.....L..NN........A.  215
GP    ..Q.........V.....I.........PVN...AKKNTGKLTDHYPVV---------...KLTEEVSAGGGSSAVQDGG.T.EGTEPVA---A.E........AM..SSG.......A.  221

AAAV  GNWHCDSQWLENGVVTRTTRTWVLPSYNNHLYKRIQGPS-GGDNNNKFFGFSTPWGYFDYNRFHCHFSPRDWQRLINNNWGIRPKAMRFRLFNIQVKEVTVQDFNTTIGNNLTSTVQVFA  350
AAV2  ........T.MGDR.I.TS....A..T.......Q.SSQ.-.AS.D.HY..Y........F.........................F...RLN.K..........QN.GT...A..........T  344
AAV4  .D.....T.S.GH.T.TS.......T........LGE----SLQS.TYN..........F.........................M......VKI........TSNGE..VA.........I..  335
AAV5  .D.....T.MGDR...KS............Q.RE.KSG.VD.S.A.AY..Y........F....S.W............Y..F..RSL.VKI............ST...A..........T  335
GP    .........MG.T.I.K.............I..A.TSGT-SQ.A.VQYA.Y........F..................H......SLK.KI..V.......T..QTK..A......I...T  340

AAAV  DKDYQLPYVLGSATEGTFPPFPADIYTIPQYGYCTLNYNNEAV---DRSAFYCLDYFPSDMLRTGNNFEFTYTFEDVPFHSMFAHNQTLDRLMNPLVDQYLWAFSSVSQA---GSSGRAL  464
AAV2  .SE..........HQ.CL......VFMV.......---LTL.NGSQAVG..S.....E....Q........T.S..........SY..S.S.........I....YYL.RTNTPSGTTTQS-R.  460
AAV4  .SS.E....MDAGQ..SL......N.VFMV......G.VTG.TSQQQT..N.....E....Q..........I..S..K......Y..S.S.........I.....GLQ.TTTGTTLNAGTATT  455
AAV5  .D.......V.NG...CL.A..PQVF.L......-ATLNRDNTENPTE..S.F..E....K...........N..E.....S..PS.N.FK.A..........YR.V.TNN-------TGGV  447
GP    .DEH...........M.....S.V.AL.......MHT.QNGARFN........E....Q..........FD..E.........S.D...............N.NE.-------D.S.NA  453

AAAV  HYSRATKTNMAAQYRNWLPGPFFRDQQIFTGASNITKNNVFSVWE-KGKQWELDNRTNLMQPGPAAATTFSGEPDRQAMQNTLAFSRTVYDQTTATTDRNQILITNEDEIRPTNSVGIDA  578
AAV2  QF.Q.GASDIRD.S.......CY.Q.RVSKTSADNNNSEYSWTGA-----TKYHLNGRDSLVN.GP.MASHKDDEEKFFPQSGVLIFGKQGSEKTNV.IEKVM..D.E...T..P.ATEQ  570
AAV4  NFTKLRP..FSNFKK......SIKQ.GFSKT.NQNY.IPATGSDSLIKYETHSTLDGRWSALT.GPPMATA.PA.SKFSNSQ.I.AGPKQNGN...VP-GTLIF.S.E.LAA..ATDT.M  569
AAV5  QFNKNLAGRY.NT.K..F...MG.T.GWNL.SGVNRASVSAFATT--NRMELEGASYQVPPQPNGMTNNLQ.SNTYALENTMIFN.QPANPG....YLEGNM...S.S.TQ.V.R.AYNV  560
GP    QFKK.V.GAYGTMG.......K.L..RVRAYTGGTDNYANWNI.S-N.NKVN.KD.QY.L....VS..YTE..ASSLPA..I.GIAKDP.RSGST.AGISD.MV.E.Q.VA...G..WKP  567

       Heparin binding
AAAV  WGAVPTNNQSIVTPGTRAAVNNQGALPGMVWQNRDIYPTGTHLAKIPDTDNHFHPSPLIGRFGCKHPPPQIFIKNTPVPANPSETFQTAKVASFINQYSTGQCTVEIFWELKKETSKRWN  698
AAV2  Y.S.S..L.RGNRQAAT.D..T..V.......D..V.LQ.PIW....H..G.......M.G..L........L............T..SA..F.....T......VS...E...Q..N.....  690
AAV4  ..NL.GGD..NSNLP.VDRLTAL..V...........YQ.PIW....H..G.........G..L.......................AT..SSTP.N...T......VS.Q.D..IQ..R.....  689
AAV5  G.QMA.....ST.APATGTY.L.EIV..S..ME..V.LQ.PIW....E.GA......AM.G..L......MML.......G.-ITS.SDVP.S...T.......V...ME.......N.....  679
GP    Y.RTV..E.NTT.AP.SSDLDVL............LQ.PIG....K..GK.....NL.G..LHN....V..........D.PVEYVHQ.WN.Y.T..........MV....R..N.....  687

AAAV  PEIQFTSNFGNAADIQFAVSDTGSYSEPRPIGTRYLTKPL  743
AAV2  ....Y...YNKSVNVD.T.DTN.V............RN.  735
AAV4  ..V.....Y.QQNSLLW.PDAA.K.T...A.........HH.  734
AAV5  ....Y.N.YNDPQFVD..PDS..E.RTT..........R..  724
GP    .........S.RTS.M..PNE..G.V.D.L.......QN.  732
```

FIG.4B

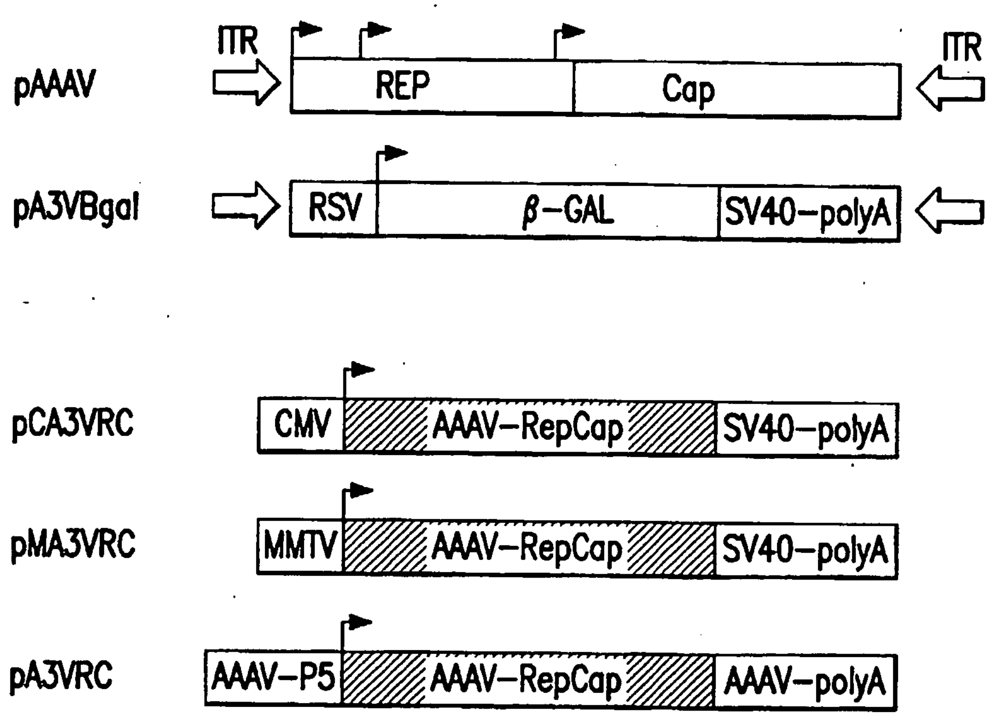

pAAAV
ITR
REP
Cap
ITR
pA3VBgal
RSV
β−GAL
SV40−polyA
pCA3VRC
CMV
AAAV−RepCap
SV40−polyA
pMA3VRC
MMTV
AAAV−RepCap
SV40−polyA
pA3VRC
AAAV−P5
AAAV−RepCap
AAAV−polyA

FIG.5A

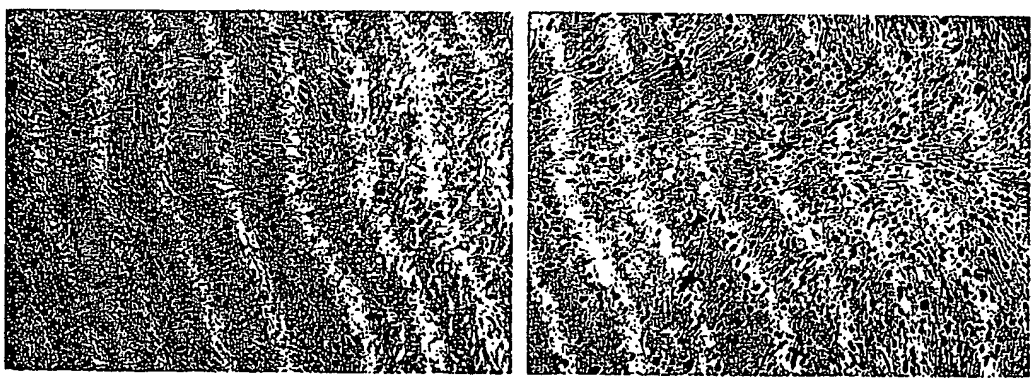
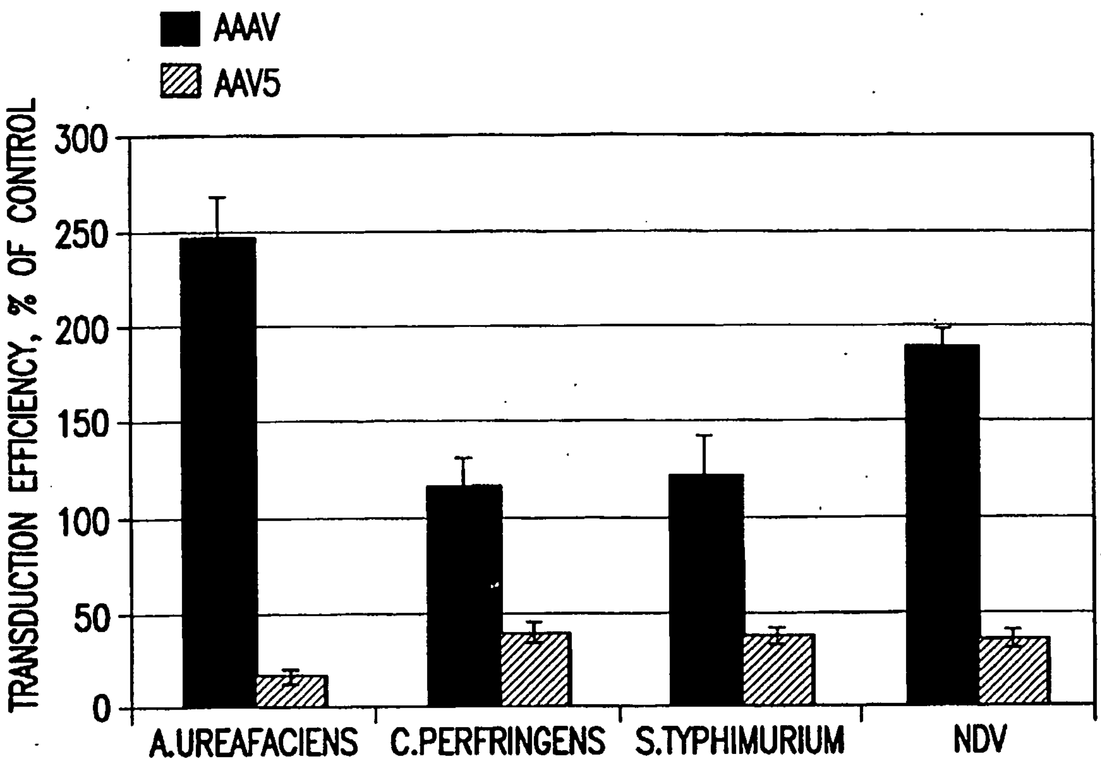


FIG.5B



AAAV
AAV5
TRANSDUCTION EFFICIENCY, % OF CONTROL
300
250
200
150
100
50
0
A.UREAFACIENS
C.PERFRINGENS
S.TYPHIMURIUM
NDV

FIG.6

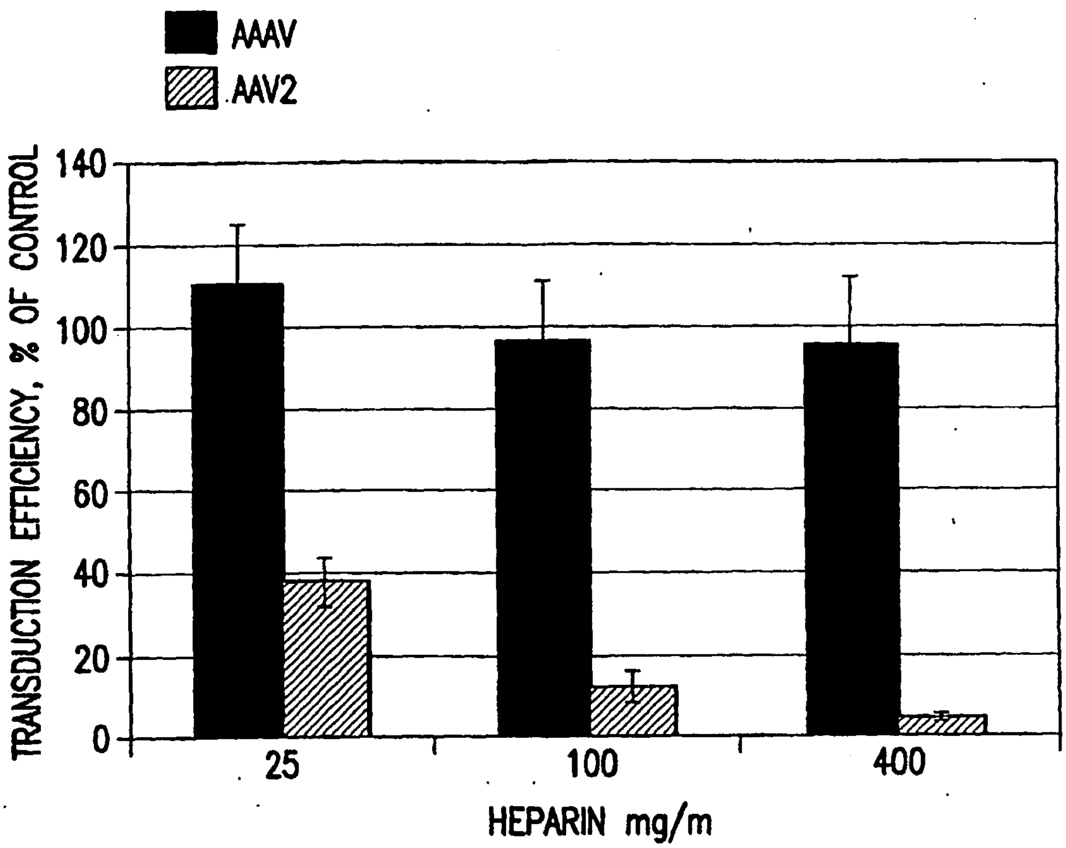

AAAV
AAV2
TRANSDUCTION EFFICIENCY, % OF CONTROL
140
120
100
80
60
40
20
0
25
100
400
HEPARIN mg/m

FIG.7

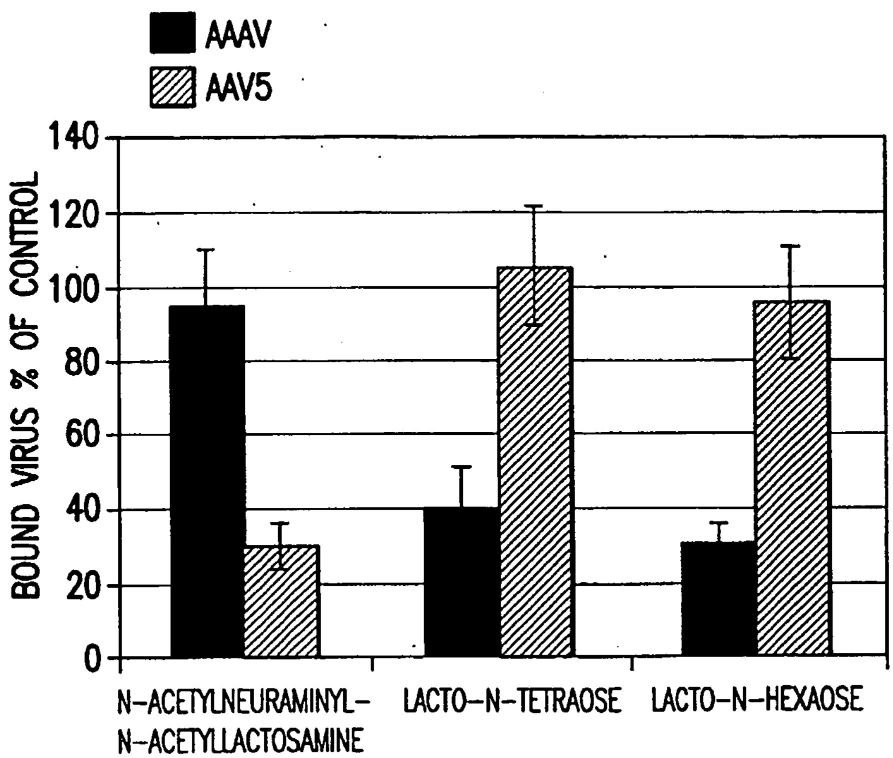
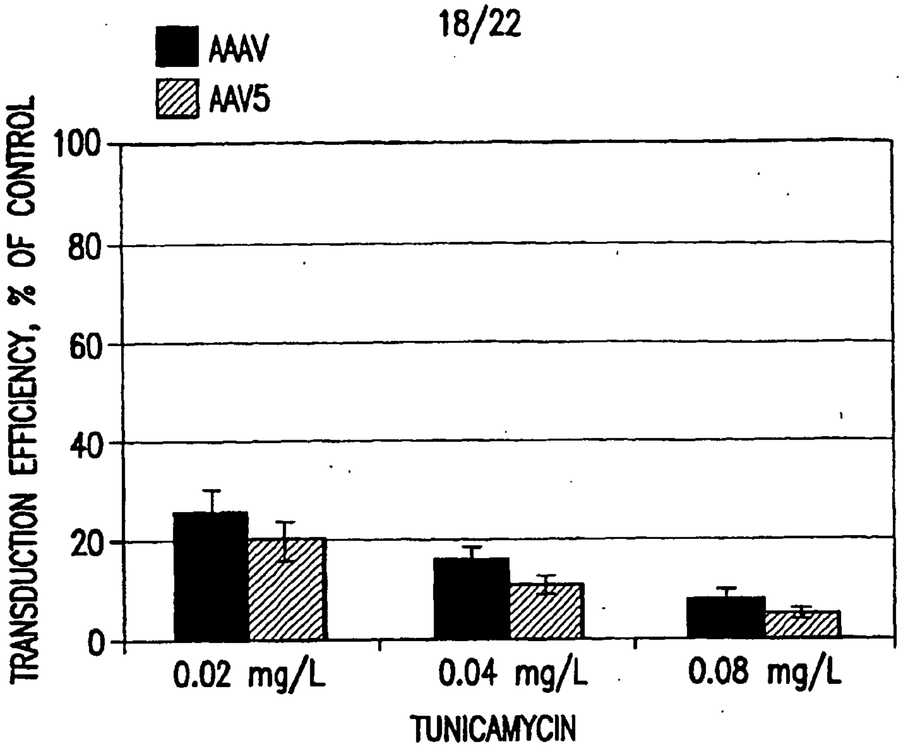
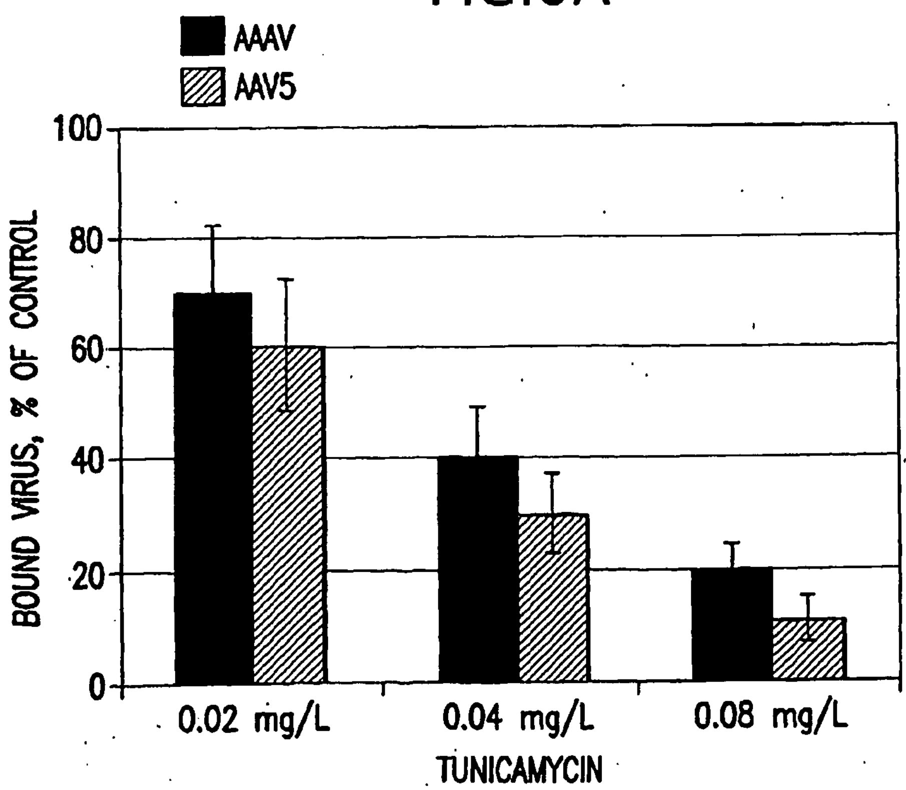

AAAV
AAV5
BOUND VIRUS % OF CONTROL
140
120
100
80
60
40
20
0
N-ACETYLNEURAMINYL-N-ACETYLLACTOSAMINE
LACTO-N-TETRAOSE
LACTO-N-HEXAOSE

FIG.8



AAAV
AAV5
TRANSDUCTION EFFICIENCY, % OF CONTROL
100
80
60
40
20
0
0.02 mg/L
0.04 mg/L
0.08 mg/L
TUNICAMYCIN

FIG.9A



AAAV
AAV5
BOUND VIRUS, % OF CONTROL
100
80
60
40
20
0
0.02 mg/L
0.04 mg/L
0.08 mg/L
TUNICAMYCIN

FIG.9B.

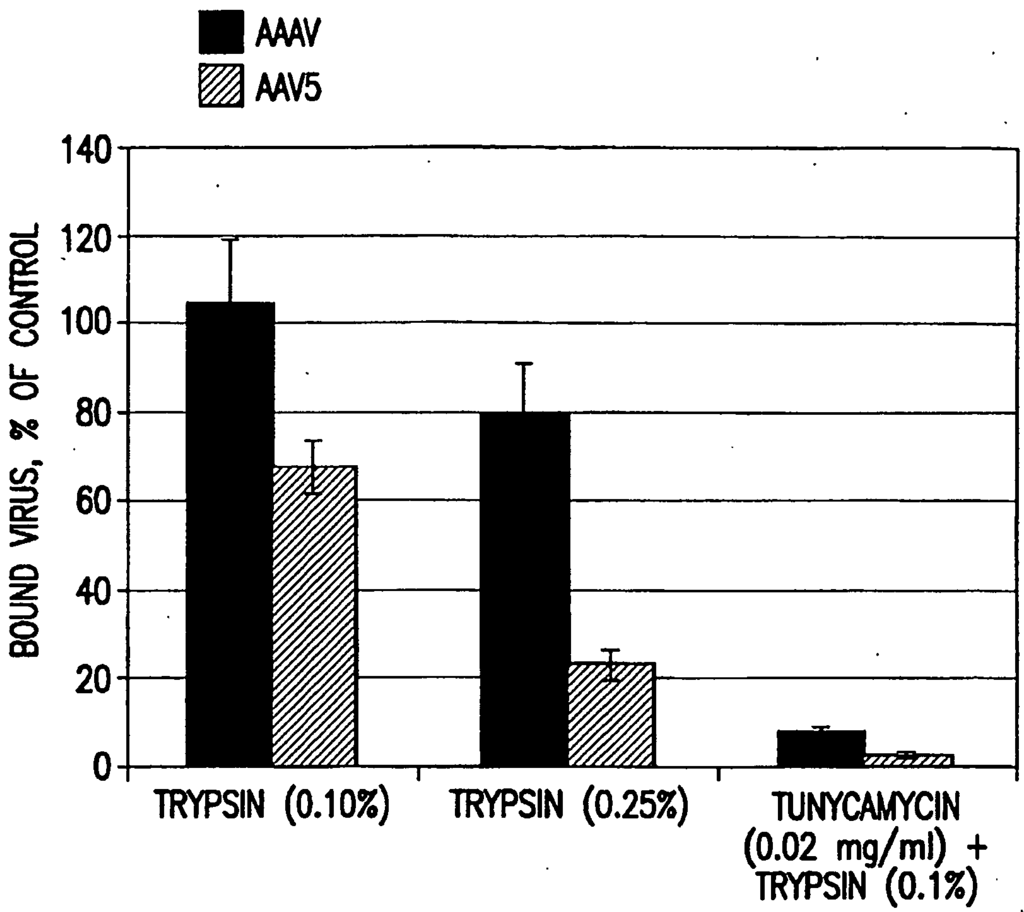

AAAV
AAV5
BOUND VIRUS, % OF CONTROL
140
120
100
80
60
40
20
0
TRYPSIN (0.10%)
TRYPSIN (0.25%)
TUNYCAMYCIN (0.02 mg/ml) + TRYPSIN (0.1%)

FIG.10

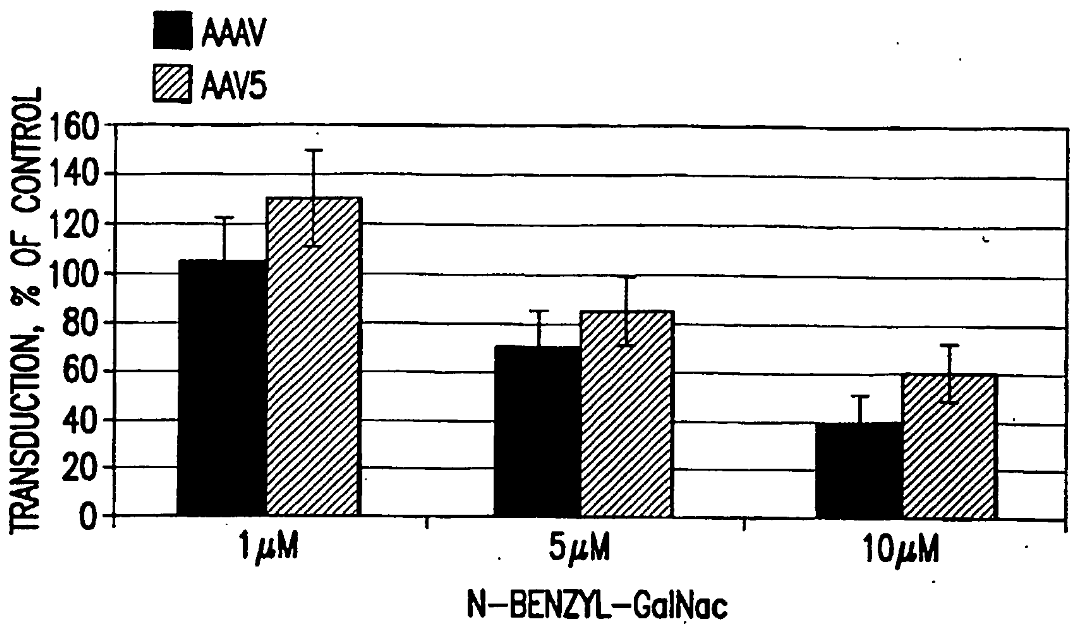


FIG.11A

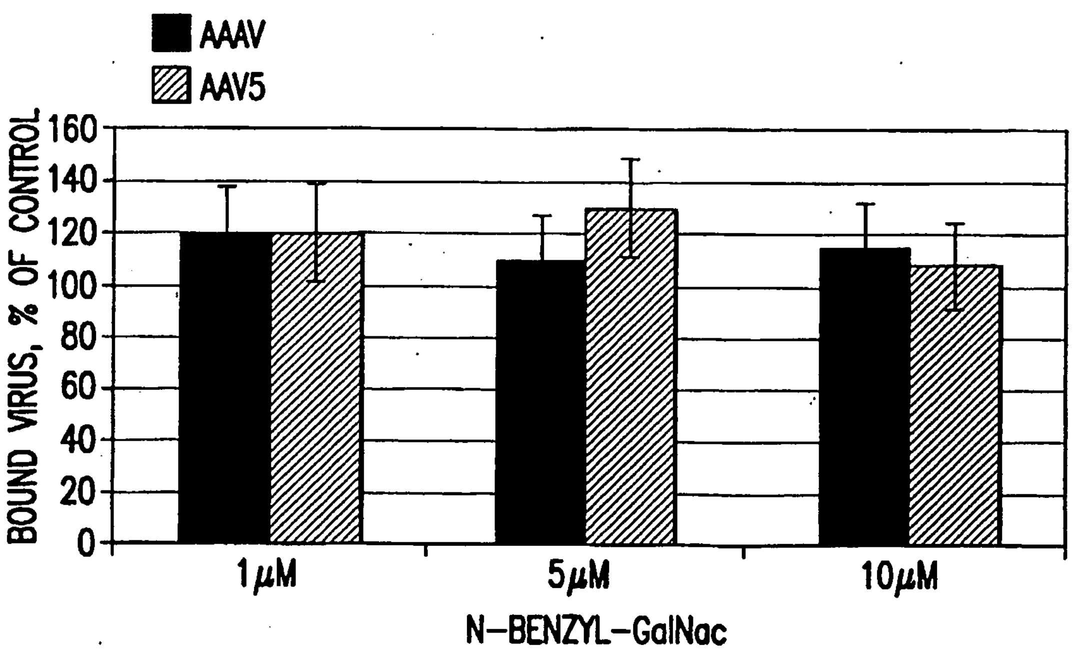


FIG.11B

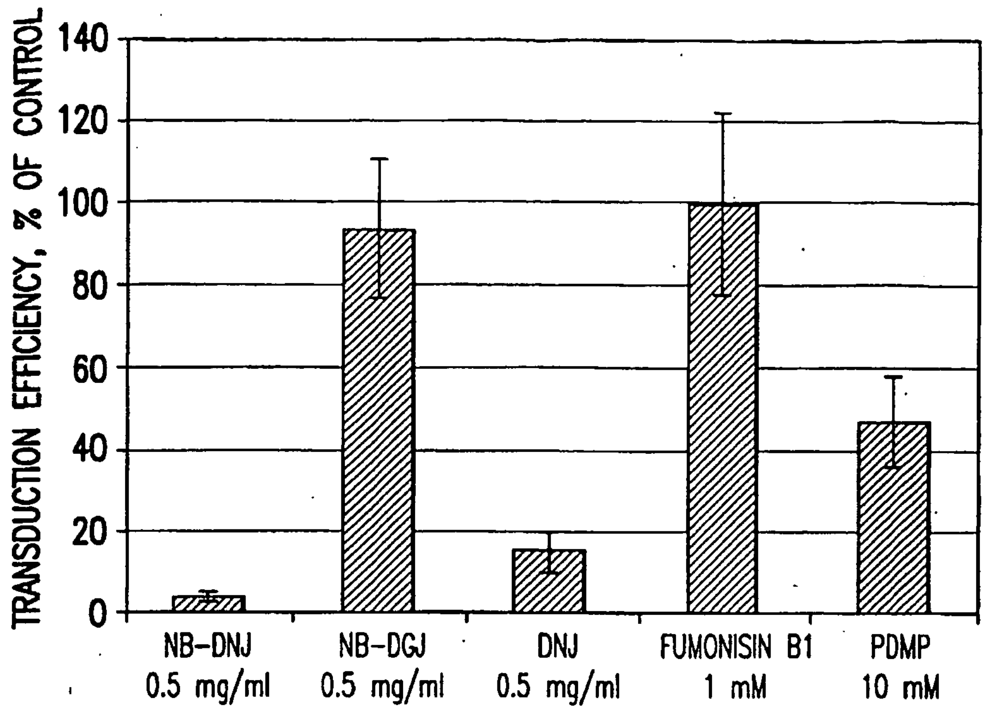


FIG.12A

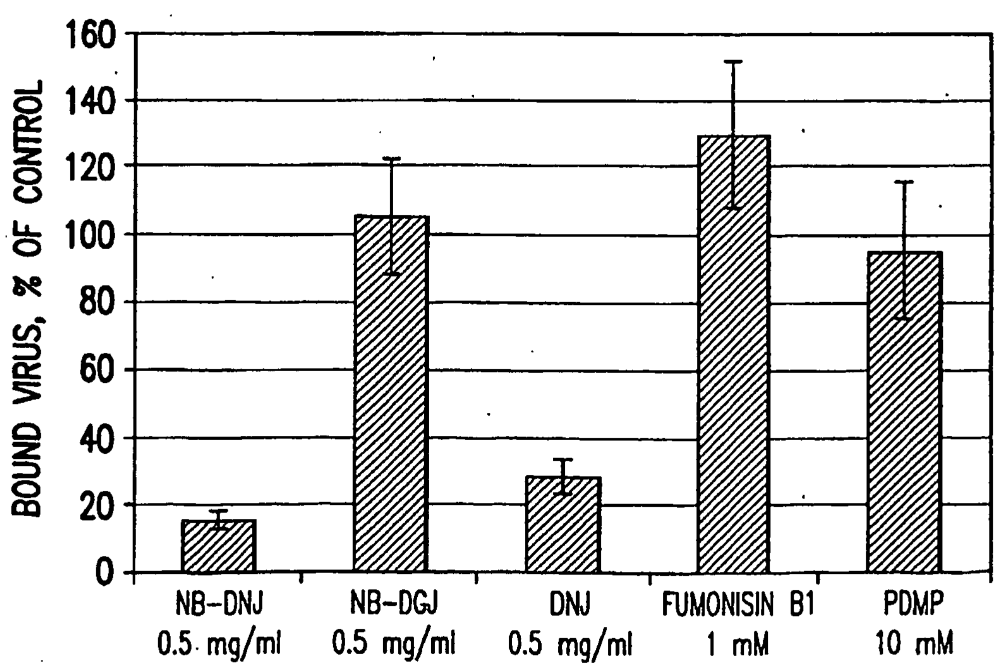


FIG.12B

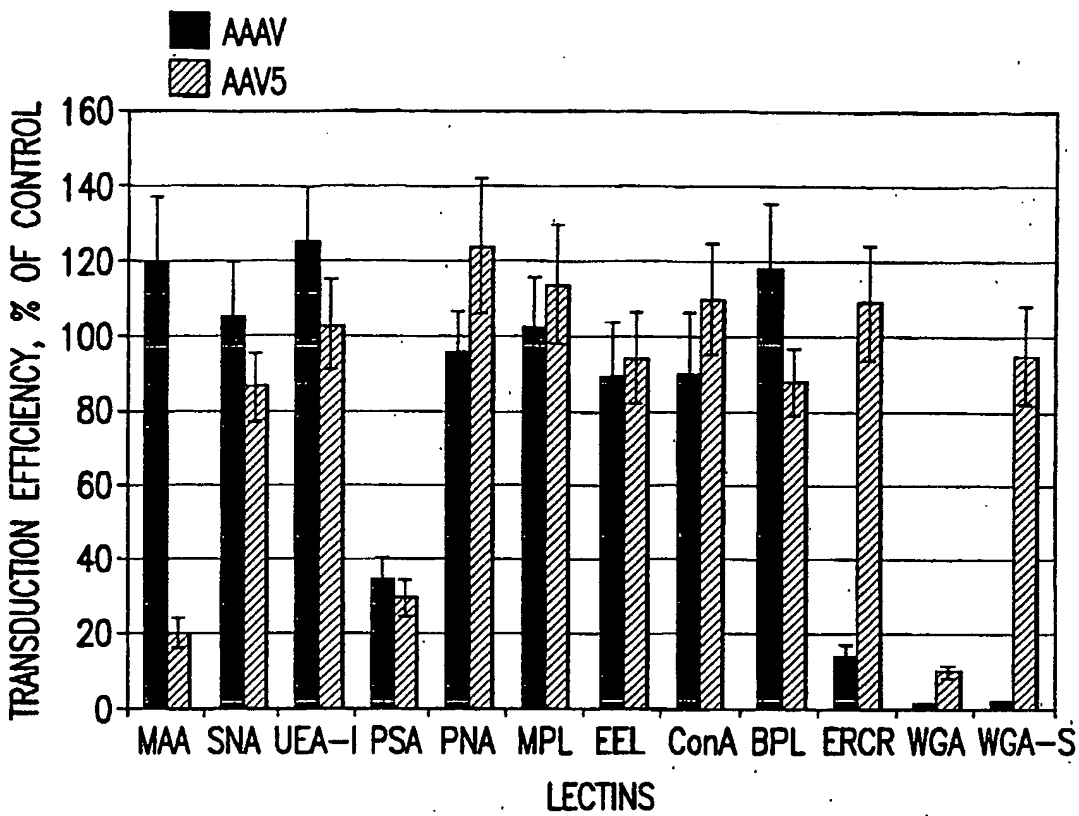


FIG.13A

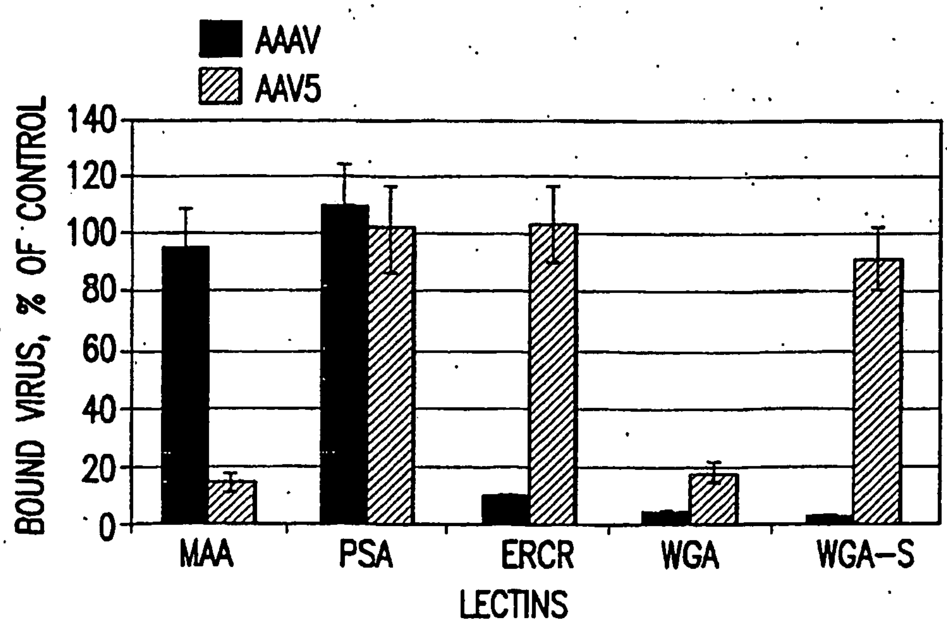


FIG.13B

SEQUENCE LISTING

<110> Government of the United States of America, as represented by the
Secretary, Department of Health & Human Services, c/o National
Institutes
of Health

<120> AVIAN ADENOASSOCIATED VIRUS (AAAV) AND
USES THEREOF

<130> 14014.0412P1

<140> Unassigned
<141> 2004-05-18

<150> 60/472,066
<151> 2003-05-19

<160> 24

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 4694
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 1
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agcgaccgct tcgcggtcgc ggcccgagtg agcgagcgag cctgtcttgg aaactggcca 120
gcactccggt gaggtaatgc cgtcacgtgg tcgggaatgg gaacgggaaa tctcgcgaga 180
acgtaaacaa atataagacg gcgccacacg gcgctgcgtc atacgcgcgc gcgcaccggc 240
gagatgaggt cgtactacga ggtcatcgtt cagctgccca acgacgtcga gagtcaggta 300
cctggaatct ccgattcgtt cgtcaactgg attacgtcgc gagaatggac gttgcctgag 360
gacgccgatt gggatttgga ccaggtcgat caagttcaac tgacgctcgg cgacaaaatc 420
caacgggaga ttcgaactca ttgggggacg atggccaaag aaccggactt tcactatttt 480
atccaactgg aacaaggtga ggtgttcttt catttacacg tcctgctgga aacgtgttcc 540
gtaaagccga tggtactcgg aagatatatc cgacatattc aacaaaaaat tgtgagtaaa 600
gtctactgcg ccacgagcct acgatggaag gatggatgcg tggtgaccaa gaccaaaaat 660
ttcgggggcg cgaacaaggt ccgggccgag tcgtatattc ccgcctacct gatcccgaaa 720
cagcaaccgg aagtgcagtg ggcgtggact aacgtgcccg agtatataaa agcgtgcttg 780
caccgagaac tgcgtgccag tctcgcgcga cttcacttcg aggaggcggg cgtctcgcaa 840
tccaaggaaa atctcgcgag aactgcagac ggcgctcccg tgatgccgac ccgcgtcagc 900
aaacgctaca tggagctcgt ggattggctc gtggagaagg ggatcaccac cgagaaggaa 960
tggctgctgg aaaacagaga aagctttcgg agctttcagg cctcgagcaa ctcggcgcgt 1020
cagatcaaga cggccctgca aggcgccatt caggagatgc ttctgaccaa gacggcggag 1080
gactacctcg tcggaaagga tcccgtctcg gacgacgaca tccgtcagaa ccgcatctac 1140
aagattctgg aactgaacca ctacgaccca gcgtacgtgg ggagtatttt ggtcgggtgg 1200
tgccagaaga aatggggcaa gcgaaacacg ctgtggctgt tcggacatgc gaccaccggc 1260
aagaccaaca tcgcggaggc tattgcccat gctgtgccgt tctatggatg cgttaactgg 1320
accaacgaga actttccgtt caacgactgc gtcgaaaaaa tgattatctg gtgggaggag 1380
ggcaaaatga ccgccaaagt ggtggaaaca gccaaggcga ttctgggagg atctcgggtg 1440
agagtggacc aaaaatgcaa agcttcggtt ccgatcgaac cgacgccggt cattattacc 1500
agtaacacca acatgtgtta tgtcatcgac gggaacacga ccacgttcga gcataagcag 1560
ccgttggagg acaggatgtt taagctcgaa ttgctgactc ggttgcctga tgactttggt 1620
aaggtgacca aacaggaggt gcgtcaattc ttcaggtggt ctcaggatca cctgacccct 1680

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gtgatcccag aattcctagt gcggaaggcg gagtctcgca aaagacccgc cccttccggg   1740
gaaggctata taagcccgac aaagcggccc gcgctcgcag agcagcagca ggcgtcggag   1800
agcgcggacc cggttcccac caggtatcgt atcaaatgct cgaaacattg cggtatggat   1860
aaaatgttgt ttccttgcca aatttgtgaa tcgatgaaca gagatattaa tatttgtgct   1920
attcataaaa cgaccgactg taaagagtgt ttccccgact acggggataa agatgatgta   1980
gaactacccc cctgtacaga acacaacgtg tctcgttgtt atcaatgtca ttcgggcgaa   2040
ttgtatcgcg tgacttcgga ctctgacgag aaacctgccc ccgagagtga tgaaggcacc   2100
gagccatcct atgctccctg cacgattcac cacctgatgg gcaagagtca cgggttagtc   2160
acttgcgcgg cgtgtcggtt gaaaaatagt acgttgcatg atgacttgga tgacggtgat   2220
ctcgaacaat aaatgattga aatgtagcca tgtctctcat ttctgatgcg attccagatt   2280
ggttggagcg gttggtcaaa aagggagtga atgctgcagc tgatttctac catttggaaa   2340
gcggtcctcc tcgtcctaag gcaaatcagc aaactcaaga atctcttgaa aaggacgatt   2400
cgagaggtct cgtgttccca ggctacaatt atctaggccc tttcaacggt ctagataaag   2460
gagaacccgt caacgaggca gacgctgccg ccttagaaca cgacaaggct tacgacctcg   2520
aaatcaagga cgggcacaac ccgtactttg agtacaacga ggccgacaga cgtttccagg   2580
aacgtctcaa agacgatacc tcctttggag gcaatttagg taaagccatc ttccaggcca   2640
aaaagagggt tctcgaaccc tttggtctgg tggaagactc aaagacggct ccgaccggag   2700
acaagcggaa aggcgaagac gaacctcgtt tgcccgacac ttcttcacag actcccaaga   2760
aaaacaagaa gcctcgcaag gaaagacctt ccggcggggc agaagatccg ggcgaaggca   2820
cctcttccaa cgctggagca gcagcacccg cctctagtgt gggatcatct atcatggctg   2880
aaggaggtgg cggcccagtg ggcgatgcag gccagggtgc cgatggagtg ggcaattcct   2940
ccggaaattg gcattgcgat tcccaatggc tggaaaacgg agtcgtcact cgaaccaccc   3000
gaacctgggt cttgcccagc tacaacaacc acctgtacaa acgaatccaa ggacccagcg   3060
gaggcgacaa caacaacaaa ttctttggat tcagcacccc ctggggatac tttgactaca   3120
atcgattcca ctgccacttt tccccgcgag actggcaacg actcatcaac aacaactggg   3180
gcatccgtcc caaagcgatg cgctttagac tctttaacat ccaggttaaa gaggtcacgg   3240
tccaagactt caacaccacc atcggcaaca acctcaccag tacggtccag gtctttgcgg   3300
acaaggacta ccaactgccg tacgtcctcg gatcggctac cgaaggcacc ttcccgccgt   3360
tcccagcgga tatctacacg atcccgcagt acgggtactg cacgctaaac tacaacaacg   3420
aggcggtgga tcgttcggcc ttctactgtc tggactactt tccctcagac atgctgcgga   3480
caggaaataa ctttgagttt acttacacct tcgaggacgt tcctttccat agcatgtttg   3540
cccacaacca gacgctagac cggctgatga atcccctcgt ggatcagtac ctctgggctt   3600
tcagctccgt cagccaagca ggctcatctg gacgagctct tcattactcg cgggcgacta   3660
aaaccaacat ggcggctcaa tataggaact ggttacctgg gcctttcttc cgtgatcagc   3720
aaatctttac gggcgctagc aacatcacta aaaataacgt ctttagcgtt tgggaaaaag   3780
gcaagcaatg ggaactcgac aatcggacca acctaatgca gcccggtcct gcggcagcga   3840
ccacctttag cggagaacct gaccgtcaag ccatgcaaaa cacgctggct tttagcagga   3900
ccgtctacga tcaaacgacc gccacgaccg atcgtaacca gatactcatc accaacgaag   3960
acgaaatcag acccaccaac tcggtcggta tcgacgcgtg ggagcagtt cccaccaaca   4020
accagtcgat cgtgaccccc ggcactcgcg cggccgtcaa caatcaaggg gcgcttcccg   4080
ggatggtgtg gcaaaacaga gacatttacc ctacagggac ccatttggcc aaaattcccg   4140
acactgacaa tcacttccat ccgtccccgc ttattgggcg gtttggctgc aagcatcccc   4200
ctccccagat tttcattaaa aacacacccg tccctgccaa cccttcggaa acgttccaga   4260
cggccaaagt ggcctccttc atcaaccagt actcgaccgg acagtgcacc gtcgaaatct   4320
tttgggaact caagaaggaa acctccaagc gctggaaccc cgaaatccag ttcacctcca   4380
actttggcaa cgcggccgac atccagtttg ccgtctccga cacgggatcc tattccgaac   4440
ctcgtcccat cggtacccgt taccttacca aacctctgta aattaaaccc ttcaataaac   4500
cgtttatgcg taactgtatt tccgtctcct gtcgttattc agtcacatga tgcggcatta   4560
cctcaccgga gtgctggcca gtttccaaga caggctcgct cgctcactcg ggccggggcc   4620
ccaaaggggc ccctagcgac cgcttcgcgg tcgcggcccg agtgagcgag cgagcctgtc   4680
ttggaaactg gcca                                                      4694
```

<210> 2
<211> 1989
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 2
atgaggtcgt actacgaggt catcgttcag ctgcccaacg acgtcgagag tcaggtacct 60
ggaatctccg attcgttcgt caactggatt acgtcgcgag aatggacgtt gcctgaggac 120
gccgattggg atttggacca ggtcgatcaa gttcaactga cgctcggcga caaaatccaa 180
cgggagattc gaactcattg ggggacgatg gccaaagaac cggactttca ctattttatc 240
caactggaac aaggtgaggt gttctttcat ttacacgtcc tgctggaaac gtgttccgta 300
aagccgatgg tactcggaag atatatccga catattcaac aaaaaattgt gagtaaagtc 360
tactgcgcca cgagcctacg atggaaggat ggatgcgtgg tgaccaagac caaaaatttc 420
gggggcgcga acaaggtccg ggccgagtcg tatattcccg cctacctgat cccgaaacag 480
caaccggaag tgcagtgggc gtggactaac gtgcccgagt atataaaagc gtgcttgcac 540
cgagaactgc gtgccagtct cgcgcgactt cacttcgagg aggcgggcgt ctcgcaatcc 600
aaggaaaatc tcgcgagaac tgcagacggc gctcccgtga tgccgacccg cgtcagcaaa 660
cgctacatgg agctcgtgga ttggctcgtg gagaagggga tcaccaccga gaaggaatgg 720
ctgctggaaa acagagaaag ctttcggagc tttcaggcct cgagcaactc ggcgcgtcag 780
atcaagacgg ccctgcaagg cgccattcag gagatgcttc tgaccaagac ggcggaggac 840
tacctcgtcg gaaaggatcc cgtctcggac gacgacatcc gtcagaaccg catctacaag 900
attctggaac tgaaccacta cgacccagcg tacgtgggga gtattttggt cgggtggtgc 960
cagaagaaat ggggcaagcg aaacacgctg tggctgttcg gacatgcgac caccggcaag 1020
accaacatcg cggaggctat tgcccatgct gtgccgttct atggatgcgt taactggacc 1080
aacgagaact ttccgttcaa cgactgcgtc gaaaaaatga ttatctggtg ggaggagggc 1140
aaaatgaccg ccaaagtggt ggaaacagcc aaggcgattc tgggaggatc tcgggtgaga 1200
gtggaccaaa aatgcaaagc ttcggttccg atcgaaccga cgccggtcat tattaccagt 1260
aacaccaaca tgtgttatgt catcgacggg aacacgacca cgttcgagca taagcagccg 1320
ttggaggaca ggatgtttaa gctcgaattg ctgactcggt tgcctgatga ctttggtaag 1380
gtgaccaaac aggaggtgcg tcaattcttc aggtggtctc aggatcacct gacccctgtg 1440
atcccagaat tcctagtgcg gaaggcggag tctcgcaaaa gacccgcccc ttccggggaa 1500
ggctatataa gcccgacaaa gcggcccgcg ctcgcagagc agcagcaggc gtcggagagc 1560
gcggacccgg ttcccaccag gtatcgtatc aaatgctcga aacattgcgg tatggataaa 1620
atgttgtttc cttgccaaat ttgtgaatcg atgaacagag atattaatat ttgtgctatt 1680
cataaaacga ccgactgtaa agagtgtttc cccgactacg gggataaaga tgatgtagaa 1740
ctacccccct gtacagaaca caacgtgtct cgttgttatc aatgtcattc gggcgaattg 1800
tatcgcgtga cttcggactc tgacgagaaa cctgccccсg agagtgatga aggcaccgag 1860
ccatcctatg ctccctgcac gattcaccac ctgatgggca agagtcacgg gttagtcact 1920
tgcgcggcgt gtcggttgaa aaatagtacg ttgcatgatg acttggatga cggtgatctc 1980
gaacaataa 1989

<210> 3
<211> 662
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 3
Met Arg Ser Tyr Tyr Glu Val Ile Val Gln Leu Pro Asn Asp Val Glu
1 5 10 15
Ser Gln Val Pro Gly Ile Ser Asp Ser Phe Val Asn Trp Ile Thr Ser
20 25 30
Arg Glu Trp Thr Leu Pro Glu Asp Ala Asp Trp Asp Leu Asp Gln Val
35 40 45
Asp Gln Val Gln Leu Thr Leu Gly Asp Lys Ile Gln Arg Glu Ile Arg
50 55 60
Thr His Trp Gly Thr Met Ala Lys Glu Pro Asp Phe His Tyr Phe Ile
65 70 75 80
Gln Leu Glu Gln Gly Glu Val Phe Phe His Leu His Val Leu Leu Glu
85 90 95
Thr Cys Ser Val Lys Pro Met Val Leu Gly Arg Tyr Ile Arg His Ile
100 105 110

```
Gln Gln Lys Ile Val Ser Lys Val Tyr Cys Ala Thr Ser Leu Arg Trp
        115                 120                 125
Lys Asp Gly Cys Val Val Thr Lys Thr Lys Asn Phe Gly Gly Ala Asn
    130                 135                 140
Lys Val Arg Ala Glu Ser Tyr Ile Pro Ala Tyr Leu Ile Pro Lys Gln
145                 150                 155                 160
Gln Pro Glu Val Gln Trp Ala Trp Thr Asn Val Pro Glu Tyr Ile Lys
                165                 170                 175
Ala Cys Leu His Arg Glu Leu Arg Ala Ser Leu Ala Arg Leu His Phe
            180                 185                 190
Glu Glu Ala Gly Val Ser Gln Ser Lys Glu Asn Leu Ala Arg Thr Ala
        195                 200                 205
Asp Gly Ala Pro Val Met Pro Thr Arg Val Ser Lys Arg Tyr Met Glu
    210                 215                 220
Leu Val Asp Trp Leu Val Glu Lys Gly Ile Thr Thr Glu Lys Glu Trp
225                 230                 235                 240
Leu Leu Glu Asn Arg Glu Ser Phe Arg Ser Phe Gln Ala Ser Ser Asn
                245                 250                 255
Ser Ala Arg Gln Ile Lys Thr Ala Leu Gln Gly Ala Ile Gln Glu Met
            260                 265                 270
Leu Leu Thr Lys Thr Ala Glu Asp Tyr Leu Val Gly Lys Asp Pro Val
        275                 280                 285
Ser Asp Asp Asp Ile Arg Gln Asn Arg Ile Tyr Lys Ile Leu Glu Leu
    290                 295                 300
Asn His Tyr Asp Pro Ala Tyr Val Gly Ser Ile Leu Val Gly Trp Cys
305                 310                 315                 320
Gln Lys Lys Trp Gly Lys Arg Asn Thr Leu Trp Leu Phe Gly His Ala
                325                 330                 335
Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
            340                 345                 350
Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
        355                 360                 365
Cys Val Glu Lys Met Ile Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
    370                 375                 380
Lys Val Val Glu Thr Ala Lys Ala Ile Leu Gly Gly Ser Arg Val Arg
385                 390                 395                 400
Val Asp Gln Lys Cys Lys Ala Ser Val Pro Ile Glu Pro Thr Pro Val
                405                 410                 415
Ile Ile Thr Ser Asn Thr Asn Met Cys Tyr Val Ile Asp Gly Asn Thr
            420                 425                 430
Thr Thr Phe Glu His Lys Gln Pro Leu Glu Asp Arg Met Phe Lys Leu
        435                 440                 445
Glu Leu Leu Thr Arg Leu Pro Asp Asp Phe Gly Lys Val Thr Lys Gln
    450                 455                 460
Glu Val Arg Gln Phe Phe Arg Trp Ser Gln Asp His Leu Thr Pro Val
465                 470                 475                 480
Ile Pro Glu Phe Leu Val Arg Lys Ala Glu Ser Arg Lys Arg Pro Ala
                485                 490                 495
Pro Ser Gly Glu Gly Tyr Ile Ser Pro Thr Lys Arg Pro Ala Leu Ala
            500                 505                 510
Glu Gln Gln Gln Ala Ser Glu Ser Ala Asp Pro Val Pro Thr Arg Tyr
        515                 520                 525
Arg Ile Lys Cys Ser Lys His Cys Gly Met Asp Lys Met Leu Phe Pro
    530                 535                 540
Cys Gln Ile Cys Glu Ser Met Asn Arg Asp Ile Asn Ile Cys Ala Ile
545                 550                 555                 560
His Lys Thr Thr Asp Cys Lys Glu Cys Phe Pro Asp Tyr Gly Asp Lys
                565                 570                 575
Asp Asp Val Glu Leu Pro Pro Cys Thr Glu His Asn Val Ser Arg Cys
            580                 585                 590
```

```
Tyr Gln Cys His Ser Gly Glu Leu Tyr Arg Val Thr Ser Asp Ser Asp
        595                 600                 605
Glu Lys Pro Ala Pro Glu Ser Asp Glu Gly Thr Glu Pro Ser Tyr Ala
    610                 615                 620
Pro Cys Thr Ile His His Leu Met Gly Lys Ser His Gly Leu Val Thr
625                 630                 635                 640
Cys Ala Ala Cys Arg Leu Lys Asn Ser Thr Leu His Asp Asp Leu Asp
                645                 650                 655
Asp Gly Asp Leu Glu Gln
            660
```

<210> 4
<211> 1323
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 4

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gaaaacagag aaagctttcg gagctttcag gcctcgagca actcggcgcg tcagatcaag     120
acggccctgc aaggcgccat tcaggagatg cttctgacca agacggcgga ggactacctc     180
gtcggaaagg atcccgtctc ggacgacgac atccgtcaga accgcatcta caagattctg     240
gaactgaacc actacgaccc agcgtacgtg ggagtatttt tggtcgggtg gtgccagaag     300
aaatggggca agcgaaacac gctgtggctg ttcggacatg cgaccaccgg caagaccaac     360
atcgcggagg ctattgccca tgctgtgccg ttctatggat gcgttaactg gaccaacgag     420
aactttccgt tcaacgactg cgtcgaaaaa atgattatct ggtgggagga gggcaaaatg     480
accgccaaag tggtggaaac agccaaggcg attctgggag gatctcgggt gagagtggac     540
caaaaatgca aagcttcggt tccgatcgaa ccgacgccgg tcattattac cagtaacacc     600
aacatgtgtt atgtcatcga cgggaacacg accacgttcg agcataagca gccgttggag     660
gacaggatgt ttaagctcga attgctgact cggttgcctg atgactttgg taaggtgacc     720
aaacaggagg tgcgtcaatt cttcaggtgg tctcaggatc acctgacccc tgtgatccca     780
gaattcctag tgcggaaggc ggagtctcgc aaaagacccg cccctccgg ggaaggctat      840
ataagcccga caaagcggcc cgcgctcgca gagcagcagc aggcgtcgga gagcgcggac     900
ccggttccca ccaggtatcg tatcaaatgc tcgaaacatt gcggtatgga taaaatgttg     960
tttccttgcc aaatttgtga atcgatgaac agagatatta atatttgtgc tattcataaa    1020
acgaccgact gtaaagagtg tttccccgac tacgggata aagatgatgt agaactaccc     1080
ccctgtacag aacacaacgt gtctcgttgt tatcaatgtc attcgggcga attgtatcgc    1140
gtgacttcgg actctgacga gaaacctgcc cccgagagtg atgaaggcac cgagccatcc    1200
tatgctccct gcacgattca ccacctgatg ggcaagagtc acgggttagt cacttgcgcg    1260
gcgtgtcggt tgaaaaatag tacgttgcat gatgacttgg atgacggtga tctcgaacaa    1320
taa                                                                  1323
```

<210> 5
<211> 440
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 5

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Met Glu Leu Val Asp Trp Leu Val Glu Lys Gly Ile Thr Thr Glu Lys
 1               5                  10                  15
Glu Trp Leu Leu Glu Asn Arg Glu Ser Phe Arg Ser Phe Gln Ala Ser
            20                  25                  30
Ser Asn Ser Ala Arg Gln Ile Lys Thr Ala Leu Gln Gly Ala Ile Gln
        35                  40                  45
```

```
Glu Met Leu Leu Thr Lys Thr Ala Glu Asp Tyr Leu Val Gly Lys Asp
    50                  55                  60
Pro Val Ser Asp Asp Asp Ile Arg Gln Asn Arg Ile Tyr Lys Ile Leu
65                  70                  75                  80
Glu Leu Asn His Tyr Asp Pro Ala Tyr Val Gly Ser Ile Leu Val Gly
                85                  90                  95
Trp Cys Gln Lys Lys Trp Gly Lys Arg Asn Thr Leu Trp Leu Phe Gly
            100                 105                 110
His Ala Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala
        115                 120                 125
Val Pro Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe
    130                 135                 140
Asn Asp Cys Val Glu Lys Met Ile Ile Trp Trp Glu Glu Gly Lys Met
145                 150                 155                 160
Thr Ala Lys Val Val Glu Thr Ala Lys Ala Ile Leu Gly Gly Ser Arg
                165                 170                 175
Val Arg Val Asp Gln Lys Cys Lys Ala Ser Val Pro Ile Glu Pro Thr
            180                 185                 190
Pro Val Ile Ile Thr Ser Asn Thr Asn Met Cys Tyr Val Ile Asp Gly
        195                 200                 205
Asn Thr Thr Thr Phe Glu His Lys Gln Pro Leu Glu Asp Arg Met Phe
    210                 215                 220
Lys Leu Glu Leu Leu Thr Arg Leu Pro Asp Asp Phe Gly Lys Val Thr
225                 230                 235                 240
Lys Gln Glu Val Arg Gln Phe Phe Arg Trp Ser Gln Asp His Leu Thr
                245                 250                 255
Pro Val Ile Pro Glu Phe Leu Val Arg Lys Ala Glu Ser Arg Lys Arg
            260                 265                 270
Pro Ala Pro Ser Gly Glu Gly Tyr Ile Ser Pro Thr Lys Arg Pro Ala
        275                 280                 285

Leu Ala Glu Gln Gln Gln Ala Ser Glu Ser Ala Asp Pro Val Pro Thr
    290                 295                 300
Arg Tyr Arg Ile Lys Cys Ser Lys His Cys Gly Met Asp Lys Met Leu
305                 310                 315                 320
Phe Pro Cys Gln Ile Cys Glu Ser Met Asn Arg Asp Ile Asn Ile Cys
                325                 330                 335
Ala Ile His Lys Thr Thr Asp Cys Lys Glu Cys Phe Pro Asp Tyr Gly
            340                 345                 350
Asp Lys Asp Asp Val Glu Leu Pro Pro Cys Thr Glu His Asn Val Ser
        355                 360                 365
Arg Cys Tyr Gln Cys His Ser Gly Glu Leu Tyr Arg Val Thr Ser Asp
    370                 375                 380
Ser Asp Glu Lys Pro Ala Pro Glu Ser Asp Glu Gly Thr Glu Pro Ser
385                 390                 395                 400
Tyr Ala Pro Cys Thr Ile His His Leu Met Gly Lys Ser His Gly Leu
                405                 410                 415
Val Thr Cys Ala Ala Cys Arg Leu Lys Asn Ser Thr Leu His Asp Asp
            420                 425                 430
Leu Asp Asp Gly Asp Leu Glu Gln
        435                 440
```

<210> 6
<211> 1626
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 6
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ggaatctccg attcgttcgt caactggatt acgtcgcgag aatggacgtt gcctgaggac 120
gccgattggg atttggacca ggtcgatcaa gttcaactga cgctcggcga caaaatccaa 180
cgggagattc gaactcattg gggacgatg gccaaagaac cggactttca ctattttatc 240
caactggaac aaggtgaggt gttctttcat ttacacgtcc tgctggaaac gtgttccgta 300
aagccgatgg tactcggaag atatatccga catattcaac aaaaaattgt gagtaaagtc 360
tactgcgcca cgagcctacg atggaaggat ggatgcgtgg tgaccaagac caaaaatttc 420
gggggcgcga acaaggtccg ggccgagtcg tatattcccg cctacctgat cccgaaacag 480
caaccggaag tgcagtgggc gtggactaac gtgcccgagt atataaaagc gtgcttgcac 540
cgagaactgc gtgccagtct cgcgcgactt cacttcgagg aggcgggcgt ctcgcaatcc 600
aaggaaaatc tcgcgagaac tgcagacggc gctcccgtga tgccgacccg cgtcagcaaa 660
cgctacatgg agctcgtgga ttggctcgtg gagaagggga tcaccaccga gaaggaatgg 720
ctgctggaaa acagagaaag ctttcggagc tttcaggcct cgagcaactc ggcgcgtcag 780
atcaagacgg ccctgcaagg cgccattcag gagatgcttc tgaccaagac ggcggaggac 840
tacctcgtcg gaaaggatcc cgtctcggac gacgacatcc gtcagaaccg catctacaag 900
attctggaac tgaaccacta cgacccagcg tacgtgggga gtattttggt cgggtggtgc 960
cagaagaaat ggggcaagcg aaacacgctg tggctgttcg gacatgcgac caccggcaag 1020
accaacatcg cggaggctat tgcccatgct gtgccgttct atggatgcgt taactggacc 1080
aacgagaact ttccgttcaa cgactgcgtc gaaaaaatga ttatctggtg ggaggagggc 1140
aaaatgaccg ccaaagtggt ggaaacagcc aaggcgattc tgggaggatc tcgggtgaga 1200
gtggaccaaa aatgcaaagc ttcggttccg atcgaaccga cgccggtcat tattaccagt 1260
aacaccaaca tgtgttatgt catcgacggg aacacgacca cgttcgagca taagcagccg 1320
ttggaggaca ggatgtttaa gctcgaattg ctgactcggt tgcctgatga ctttggtaag 1380
gtgaccaaac aggaggtgcg tcaattcttc aggtggtctc aggatcacct gacccctgtg 1440
atcccagaat tcctagtgcg gaaggcggag tctcgcaaaa gacccgcccc ttccggggaa 1500
ggctatataa gcccgacaaa gcggcccgcg ctcgcagagc agcagcaggc gtcggagagc 1560
gcggacccgg ttcccaccag attggttgga gcggttggtc aaaaagggag tgaatgctgc 1620
agctga 1626

<210> 7
<211> 541
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 7
Met Arg Ser Tyr Tyr Glu Val Ile Val Gln Leu Pro Asn Asp Val Glu
1 5 10 15
Ser Gln Val Pro Gly Ile Ser Asp Ser Phe Val Asn Trp Ile Thr Ser
20 25 30
Arg Glu Trp Thr Leu Pro Glu Asp Ala Asp Trp Asp Leu Asp Gln Val
35 40 45
Asp Gln Val Gln Leu Thr Leu Gly Asp Lys Ile Gln Arg Glu Ile Arg
50 55 60
Thr His Trp Gly Thr Met Ala Lys Glu Pro Asp Phe His Tyr Phe Ile
65 70 75 80
Gln Leu Glu Gln Gly Glu Val Phe Phe His Leu His Val Leu Leu Glu
85 90 95
Thr Cys Ser Val Lys Pro Met Val Leu Gly Arg Tyr Ile Arg His Ile
100 105 110
Gln Gln Lys Ile Val Ser Lys Val Tyr Cys Ala Thr Ser Leu Arg Trp
115 120 125
Lys Asp Gly Cys Val Val Thr Lys Thr Lys Asn Phe Gly Gly Ala Asn
130 135 140
Lys Val Arg Ala Glu Ser Tyr Ile Pro Ala Tyr Leu Ile Pro Lys Gln
145 150 155 160

```
Gln Pro Glu Val Gln Trp Ala Trp Thr Asn Val Pro Glu Tyr Ile Lys

                    165                 170                 175
Ala Cys Leu His Arg Glu Leu Arg Ala Ser Leu Ala Arg Leu His Phe
            180                 185                 190
Glu Glu Ala Gly Val Ser Gln Ser Lys Glu Asn Leu Ala Arg Thr Ala
        195                 200                 205
Asp Gly Ala Pro Val Met Pro Thr Arg Val Ser Lys Arg Tyr Met Glu
    210                 215                 220
Leu Val Asp Trp Leu Val Glu Lys Gly Ile Thr Thr Glu Lys Glu Trp
225                 230                 235                 240
Leu Leu Glu Asn Arg Glu Ser Phe Arg Ser Phe Gln Ala Ser Ser Asn
                245                 250                 255
Ser Ala Arg Gln Ile Lys Thr Ala Leu Gln Gly Ala Ile Gln Glu Met
            260                 265                 270
Leu Leu Thr Lys Thr Ala Glu Asp Tyr Leu Val Gly Lys Asp Pro Val
        275                 280                 285
Ser Asp Asp Asp Ile Arg Gln Asn Arg Ile Tyr Lys Ile Leu Glu Leu
    290                 295                 300
Asn His Tyr Asp Pro Ala Tyr Val Gly Ser Ile Leu Val Gly Trp Cys
305                 310                 315                 320
Gln Lys Lys Trp Gly Lys Arg Asn Thr Leu Trp Leu Phe Gly His Ala
                325                 330                 335
Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
            340                 345                 350
Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
        355                 360                 365
Cys Val Glu Lys Met Ile Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
    370                 375                 380
Lys Val Val Glu Thr Ala Lys Ala Ile Leu Gly Gly Ser Arg Val Arg
385                 390                 395                 400
Val Asp Gln Lys Cys Lys Ala Ser Val Pro Ile Glu Pro Thr Pro Val
                405                 410                 415
Ile Ile Thr Ser Asn Thr Asn Met Cys Tyr Val Ile Asp Gly Asn Thr
            420                 425                 430
Thr Thr Phe Glu His Lys Gln Pro Leu Glu Asp Arg Met Phe Lys Leu
        435                 440                 445
Glu Leu Leu Thr Arg Leu Pro Asp Asp Phe Gly Lys Val Thr Lys Gln
    450                 455                 460
Glu Val Arg Gln Phe Phe Arg Trp Ser Gln Asp His Leu Thr Pro Val
465                 470                 475                 480
Ile Pro Glu Phe Leu Val Arg Lys Ala Glu Ser Arg Lys Arg Pro Ala
                485                 490                 495
Pro Ser Gly Glu Gly Tyr Ile Ser Pro Thr Lys Arg Pro Ala Leu Ala
            500                 505                 510
Glu Gln Gln Gln Ala Ser Glu Ser Ala Asp Pro Val Pro Thr Arg Leu
        515                 520                 525
Val Gly Ala Val Gly Gln Lys Gly Ser Glu Cys Cys Ser
    530                 535                 540
```

<210> 8
<211> 960
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 8
atggagctcg tggattggct cgtggagaag ggatcacca ccgagaagga atggctgctg 60
gaaaacagag aaagctttcg gagctttcag gcctcgagca actcggcgcg tcagatcaag 120
acggccctgc aaggcgccat tcaggagatg cttctgacca agacggcgga ggactacctc 180
gtcggaaagg atcccgtctc ggacgacgac atccgtcaga accgcatcta caagattctg 240
gaactgaacc actacgaccc agcgtacgtg gggagtattt tggtcgggtg gtgccagaag 300
aaatggggca agcgaaacac gctgtggctg ttcggacatg cgaccaccgg caagaccaac 360
atcgcggagg ctattgccca tgctgtgccg ttctatggat gcgttaactg gaccaacgag 420
aactttccgt tcaacgactg cgtcgaaaaa atgattatct ggtgggagga gggcaaaatg 480
accgccaaag tggtggaaac agccaaggcg attctgggag gatctcgggt gagagtggac 540
caaaaatgca aagcttcggt tccgatcgaa ccgacgccgg tcattattac cagtaacacc 600
aacatgtgtt atgtcatcga cgggaacacg accacgttcg agcataagca gccgttggag 660
gacaggatgt ttaagctcga attgctgact cggttgcctg atgactttgg taaggtgacc 720
aaacaggagg tgcgtcaatt cttcaggtgg tctcaggatc acctgacccc tgtgatccca 780
gaattcctag tgcggaaggc ggagtctcgc aaaagacccg ccccttccgg ggaaggctat 840
ataagcccga caaagcggcc cgcgctcgca gagcagcagc aggcgtcgga gagcgcggac 900
ccggttccca ccagattggt tggagcggtt ggtcaaaaag ggagtgaatg ctgcagctga 960

<210> 9
<211> 319
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 9
Met Glu Leu Val Asp Trp Leu Val Glu Lys Gly Ile Thr Thr Glu Lys
1 5 10 15
Glu Trp Leu Leu Glu Asn Arg Glu Ser Phe Arg Ser Phe Gln Ala Ser
20 25 30
Ser Asn Ser Ala Arg Gln Ile Lys Thr Ala Leu Gln Gly Ala Ile Gln
35 40 45
Glu Met Leu Leu Thr Lys Thr Ala Glu Asp Tyr Leu Val Gly Lys Asp
50 55 60
Pro Val Ser Asp Asp Asp Ile Arg Gln Asn Arg Ile Tyr Lys Ile Leu
65 70 75 80
Glu Leu Asn His Tyr Asp Pro Ala Tyr Val Gly Ser Ile Leu Val Gly
85 90 95
Trp Cys Gln Lys Lys Trp Gly Lys Arg Asn Thr Leu Trp Leu Phe Gly
100 105 110
His Ala Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala
115 120 125
Val Pro Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe
130 135 140
Asn Asp Cys Val Glu Lys Met Ile Ile Trp Trp Glu Glu Gly Lys Met
145 150 155 160
Thr Ala Lys Val Val Glu Thr Ala Lys Ala Ile Leu Gly Gly Ser Arg
165 170 175
Val Arg Val Asp Gln Lys Cys Lys Ala Ser Val Pro Ile Glu Pro Thr
180 185 190
Pro Val Ile Ile Thr Ser Asn Thr Asn Met Cys Tyr Val Ile Asp Gly
195 200 205
Asn Thr Thr Thr Phe Glu His Lys Gln Pro Leu Glu Asp Arg Met Phe
210 215 220
Lys Leu Glu Leu Leu Thr Arg Leu Pro Asp Asp Phe Gly Lys Val Thr
225 230 235 240
Lys Gln Glu Val Arg Gln Phe Phe Arg Trp Ser Gln Asp His Leu Thr
245 250 255

Pro Val Ile Pro Glu Phe Leu Val Arg Lys Ala Glu Ser Arg Lys Arg
260 265 270
Pro Ala Pro Ser Gly Glu Gly Tyr Ile Ser Pro Thr Lys Arg Pro Ala
275 280 285
Leu Ala Glu Gln Gln Gln Ala Ser Glu Ser Ala Asp Pro Val Pro Thr
290 295 300
Arg Leu Val Gly Ala Val Gly Gln Lys Gly Ser Glu Cys Cys Ser
305 310 315

<210> 10
<211> 2232
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 10
atgtctctca tttctgatgc gattccagat tggttggagc ggttggtcaa aaagggagtg 60
aatgctgcag ctgatttcta ccatttggaa agcggtcctc ctcgtcctaa ggcaaatcag 120
caaactcaag aatctcttga aaaggacgat tcgagaggtc tcgtgttccc aggctacaat 180
tatctaggcc ctttcaacgg tctagataaa ggagaacccg tcaacgaggc agacgctgcc 240
gccttagaac acgacaaggc ttacgacctc gaaatcaagg acgggcacaa cccgtacttt 300
gagtacaacg aggccgacag acgtttccag gaacgtctca aagacgatac ctcctttgga 360
ggcaatttag gtaaagccat cttccaggcc aaaaagaggg ttctcgaacc ctttggtctg 420
gtggaagact caaagacggc tccgaccgga gacaagcgga aaggcgaaga cgaacctcgt 480
ttgcccgaca cttcttcaca gactcccaag aaaaacaaga agcctcgcaa ggaaagacct 540
tccggcgggg cagaagatcc gggcgaaggc acctcttcca acgctggagc agcagcaccc 600
gcctctagtg tgggatcatc tatcatggct gaaggaggtg gcggcccagt gggcgatgca 660
ggccagggtg ccgatggagt gggcaattcc tccggaaatt ggcattgcga ttcccaatgg 720
ctggaaaacg gagtcgtcac tcgaaccacc cgaacctggg tcttgcccag ctacaacaac 780
cacctgtaca aacgaatcca aggacccagc ggaggcgaca acaacaacaa attctttgga 840
ttcagcaccc cctggggata ctttgactac aatcgattcc actgccactt ttccccgcga 900
gactggcaac gactcatcaa caacaactgg ggcatccgtc ccaaagcgat gcgctttaga 960
ctctttaaca tccaggttaa agaggtcacg gtccaagact tcaacaccac catcggcaac 1020
aacctcacca gtacggtcca ggtctttgcg gacaaggact accaactgcc gtacgtcctc 1080
ggatcggcta ccgaaggcac cttcccgccg ttcccagcgg atatctacac gatcccgcag 1140
tacgggtact gcacgctaaa ctacaacaac gaggcggtgg atcgttcggc cttctactgt 1200
ctggactact ttccctcaga catgctgcgg acaggaaata actttgagtt tacttacacc 1260
ttcgaggacg ttcctttcca tagcatgttt gcccacaacc agacgctaga ccggctgatg 1320
aatcccctcg tggatcagta cctctgggct ttcagctccg tcagccaagc aggctcatct 1380
ggacgagctc ttcattactc gcgggcgact aaaaccaaca tggcggctca atataggaac 1440
tggttacctg ggcctttctt ccgtgatcag caaatcttta cgggcgctag caacatcact 1500
aaaaataacg tctttagcgt ttgggaaaaa ggcaagcaat gggaactcga caatcggacc 1560
aacctaatgc agcccggtcc tgcggcagcg accaccttta gcggagaacc tgaccgtcaa 1620
gccatgcaaa acacgctggc ttttagcagg accgtctacg atcaaacgac cgccacgacc 1680
gatcgtaacc agatactcat caccaacgaa gacgaaatca gacccaccaa ctcggtcggt 1740
atcgacgcgt ggggagcagt tcccaccaac aaccagtcga tcgtgacccc cggcactcgc 1800
gcggccgtca acaatcaagg ggcgcttccc gggatggtgt ggcaaaacag agacatttac 1860
cctacaggga cccatttggc caaaattccc gacactgaca atcacttcca tccgtccccg 1920
cttattgggc ggtttggctg caagcatccc cctccccaga ttttcattaa aaacacaccc 1980
gtccctgcca acccttcgga aacgttccag acggccaaag tggcctcctt catcaaccag 2040
tactcgaccg gacagtgcac cgtcgaaatc ttttgggaac tcaagaagga aacctccaag 2100
cgctggaacc ccgaaatcca gttcacctcc aactttggca acgcggccga catccagttt 2160
gccgtctccg acacgggatc ctattccgaa cctcgtccca tcggtacccg ttaccttacc 2220
aaacctctgt aa 2232

<210> 11
<211> 743
<212> PRT

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 11

```
Met Ser Leu Ile Ser Asp Ala Ile Pro Asp Trp Leu Glu Arg Leu Val
 1               5                  10                  15
Lys Lys Gly Val Asn Ala Ala Ala Asp Phe Tyr His Leu Glu Ser Gly
            20                  25                  30
Pro Pro Arg Pro Lys Ala Asn Gln Gln Thr Gln Glu Ser Leu Glu Lys
        35                  40                  45
Asp Asp Ser Arg Gly Leu Val Phe Pro Gly Tyr Asn Tyr Leu Gly Pro
    50                  55                  60
Phe Asn Gly Leu Asp Lys Gly Glu Pro Val Asn Glu Ala Asp Ala Ala
65                  70                  75                  80
Ala Leu Glu His Asp Lys Ala Tyr Asp Leu Glu Ile Lys Asp Gly His
                85                  90                  95
Asn Pro Tyr Phe Glu Tyr Asn Glu Ala Asp Arg Arg Phe Gln Glu Arg
            100                 105                 110
Leu Lys Asp Asp Thr Ser Phe Gly Gly Asn Leu Gly Lys Ala Ile Phe
        115                 120                 125
Gln Ala Lys Lys Arg Val Leu Glu Pro Phe Gly Leu Val Glu Asp Ser
    130                 135                 140
Lys Thr Ala Pro Thr Gly Asp Lys Arg Lys Gly Glu Asp Glu Pro Arg
145                 150                 155                 160
Leu Pro Asp Thr Ser Ser Gln Thr Pro Lys Lys Asn Lys Lys Pro Arg
                165                 170                 175
Lys Glu Arg Pro Ser Gly Gly Ala Glu Asp Pro Gly Glu Gly Thr Ser
            180                 185                 190
Ser Asn Ala Gly Ala Ala Ala Pro Ala Ser Ser Val Gly Ser Ser Ile
        195                 200                 205

Met Ala Glu Gly Gly Gly Gly Pro Val Gly Asp Ala Gly Gln Gly Ala
    210                 215                 220
Asp Gly Val Gly Asn Ser Ser Gly Asn Trp His Cys Asp Ser Gln Trp
225                 230                 235                 240
Leu Glu Asn Gly Val Val Thr Arg Thr Thr Arg Thr Trp Val Leu Pro
                245                 250                 255
Ser Tyr Asn Asn His Leu Tyr Lys Arg Ile Gln Gly Pro Ser Gly Gly
            260                 265                 270
Asp Asn Asn Asn Lys Phe Phe Gly Phe Ser Thr Pro Trp Gly Tyr Phe
        275                 280                 285
Asp Tyr Asn Arg Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg
    290                 295                 300
Leu Ile Asn Asn Asn Trp Gly Ile Arg Pro Lys Ala Met Arg Phe Arg
305                 310                 315                 320
Leu Phe Asn Ile Gln Val Lys Glu Val Thr Val Gln Asp Phe Asn Thr
                325                 330                 335
Thr Ile Gly Asn Asn Leu Thr Ser Thr Val Gln Val Phe Ala Asp Lys
            340                 345                 350
Asp Tyr Gln Leu Pro Tyr Val Leu Gly Ser Ala Thr Glu Gly Thr Phe
        355                 360                 365
Pro Pro Phe Pro Ala Asp Ile Tyr Thr Ile Pro Gln Tyr Gly Tyr Cys
    370                 375                 380
Thr Leu Asn Tyr Asn Asn Glu Ala Val Asp Arg Ser Ala Phe Tyr Cys
385                 390                 395                 400
Leu Asp Tyr Phe Pro Ser Asp Met Leu Arg Thr Gly Asn Asn Phe Glu
                405                 410                 415
```

```
Phe Thr Tyr Thr Phe Glu Asp Val Pro Phe His Ser Met Phe Ala His
            420                 425                 430
Asn Gln Thr Leu Asp Arg Leu Met Asn Pro Leu Val Asp Gln Tyr Leu
        435                 440                 445
Trp Ala Phe Ser Ser Val Ser Gln Ala Gly Ser Ser Gly Arg Ala Leu
    450                 455                 460
His Tyr Ser Arg Ala Thr Lys Thr Asn Met Ala Ala Gln Tyr Arg Asn
465                 470                 475                 480
Trp Leu Pro Gly Pro Phe Phe Arg Asp Gln Gln Ile Phe Thr Gly Ala
                485                 490                 495
Ser Asn Ile Thr Lys Asn Asn Val Phe Ser Val Trp Glu Lys Gly Lys
            500                 505                 510
Gln Trp Glu Leu Asp Asn Arg Thr Asn Leu Met Gln Pro Gly Pro Ala
        515                 520                 525
Ala Ala Thr Thr Phe Ser Gly Glu Pro Asp Arg Gln Ala Met Gln Asn
    530                 535                 540
Thr Leu Ala Phe Ser Arg Thr Val Tyr Asp Gln Thr Thr Ala Thr Thr
545                 550                 555                 560
Asp Arg Asn Gln Ile Leu Ile Thr Asn Glu Asp Glu Ile Arg Pro Thr
                565                 570                 575
Asn Ser Val Gly Ile Asp Ala Trp Gly Ala Val Pro Thr Asn Asn Gln
            580                 585                 590
Ser Ile Val Thr Pro Gly Thr Arg Ala Ala Val Asn Asn Gln Gly Ala
        595                 600                 605
Leu Pro Gly Met Val Trp Gln Asn Arg Asp Ile Tyr Pro Thr Gly Thr
    610                 615                 620
His Leu Ala Lys Ile Pro Asp Thr Asp Asn His Phe His Pro Ser Pro
625                 630                 635                 640
Leu Ile Gly Arg Phe Gly Cys Lys His Pro Pro Pro Gln Ile Phe Ile
                645                 650                 655
Lys Asn Thr Pro Val Pro Ala Asn Pro Ser Glu Thr Phe Gln Thr Ala
            660                 665                 670
Lys Val Ala Ser Phe Ile Asn Gln Tyr Ser Thr Gly Gln Cys Thr Val
        675                 680                 685
Glu Ile Phe Trp Glu Leu Lys Lys Glu Thr Ser Lys Arg Trp Asn Pro
    690                 695                 700
Glu Ile Gln Phe Thr Ser Asn Phe Gly Asn Ala Ala Asp Ile Gln Phe
705                 710                 715                 720
Ala Val Ser Asp Thr Gly Ser Tyr Ser Glu Pro Arg Pro Ile Gly Thr
                725                 730                 735
Arg Tyr Leu Thr Lys Pro Leu
            740
```

<210> 12
<211> 1797
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 12

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acggctccga ccggagacaa gcggaaaggc gaagacgaac ctcgtttgcc cgacacttct      60
tcacagactc ccaagaaaaa caagaagcct cgcaaggaaa gaccttccgg cggggcagaa     120
gatccgggcg aaggcacctc ttccaacgct ggagcagcag caccgcctc tagtgtggga     180
tcatctatca tggctgaagg aggtggcggc ccagtgggcg atgcaggcca gggtgccgat     240
ggagtgggca attcctccgg aaattggcat tgcgattccc aatggctgga aaacggagtc     300
gtcactcgaa ccacccgaac ctgggtcttg cccagctaca acaaccacct gtacaaacga     360
atccaaggac ccagcggagg cgacaacaac aacaaattct ttggattcag cacccccctgg     420
ggatactttg actacaatcg attccactgc cacttttccc cgcgagactg gcaacgactc     480
```

```
atcaacaaca actggggcat ccgtcccaaa gcgatgcgct ttagactctt taacatccag      540
gttaaagagg tcacggtcca agacttcaac accaccatcg gcaacaacct caccagtacg      600
gtccaggtct ttgcggacaa ggactaccaa ctgccgtacg tcctcggatc ggctaccgaa      660
ggcaccttcc cgccgttccc agcggatatc tacacgatcc cgcagtacgg gtactgcacg      720
ctaaactaca acaacgaggc ggtggatcgt tcggccttct actgtctgga ctactttccc      780
tcagacatgc tgcggacagg aaataacttt gagtttactt acaccttcga ggacgttcct      840
ttccatagca tgtttgccca caaccagacg ctagaccggc tgatgaatcc cctcgtggat      900
cagtacctct gggctttcag ctccgtcagc caagcaggct catctggacg agctcttcat      960
tactcgcggg cgactaaaac caacatggcg gctcaatata ggaactggtt acctgggcct     1020
ttcttccgtg atcagcaaat ctttacgggc gctagcaaca tcactaaaaa taacgtcttt     1080
agcgtttggg aaaaaggcaa gcaatgggaa ctcgacaatc ggaccaacct aatgcagccc     1140
ggtcctgcgg cagcgaccac ctttagcgga gaacctgacc gtcaagccat gcaaaacacg     1200
ctggctttta gcaggaccgt ctacgatcaa acgaccgcca cgaccgatcg taaccagata     1260
ctcatcacca acgaagacga aatcagaccc accaactcgg tcggtatcga cgcgtgggga     1320
gcagttccca ccaacaacca gtcgatcgtg acccccggca ctcgcgcggc cgtcaacaat     1380
caaggggcgc ttcccgggat ggtgtggcaa aacagagaca tttaccctac agggacccat     1440
ttggccaaaa ttcccgacac tgacaatcac ttccatccgt ccccgcttat tgggcggttt     1500
ggctgcaagc atcccccctcc ccagattttc attaaaaaca cacccgtccc tgccaaccct     1560
tcggaaacgt tccagacggc caaagtggcc tccttcatca accagtactc gaccggacag     1620
tgcaccgtcg aaatcttttg ggaactcaag aaggaaacct ccaagcgctg gaaccccgaa     1680
atccagttca cctccaactt tggcaacgcg gccgacatcc agtttgccgt ctccgacacg     1740
ggatcctatt ccgaacctcg tcccatcggt acccgttacc ttaccaaacc tctgtaa        1797
```

<210> 13
<211> 598
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 13

```
Thr Ala Pro Thr Gly Asp Lys Arg Lys Gly Glu Asp Glu Pro Arg Leu
1               5                   10                  15
Pro Asp Thr Ser Ser Gln Thr Pro Lys Lys Asn Lys Lys Pro Arg Lys
            20                  25                  30
Glu Arg Pro Ser Gly Gly Ala Glu Asp Pro Gly Glu Gly Thr Ser Ser
        35                  40                  45
Asn Ala Gly Ala Ala Ala Pro Ala Ser Ser Val Gly Ser Ser Ile Met
    50                  55                  60
Ala Glu Gly Gly Gly Gly Pro Val Gly Asp Ala Gly Gln Gly Ala Asp
65                  70                  75                  80
Gly Val Gly Asn Ser Ser Gly Asn Trp His Cys Asp Ser Gln Trp Leu
                85                  90                  95
Glu Asn Gly Val Val Thr Arg Thr Thr Arg Thr Trp Val Leu Pro Ser
            100                 105                 110
Tyr Asn Asn His Leu Tyr Lys Arg Ile Gln Gly Pro Ser Gly Gly Asp
        115                 120                 125
Asn Asn Asn Lys Phe Phe Gly Phe Ser Thr Pro Trp Gly Tyr Phe Asp
    130                 135                 140
Tyr Asn Arg Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu
145                 150                 155                 160
Ile Asn Asn Asn Trp Gly Ile Arg Pro Lys Ala Met Arg Phe Arg Leu
                165                 170                 175
Phe Asn Ile Gln Val Lys Glu Val Thr Val Gln Asp Phe Asn Thr Thr
            180                 185                 190
Ile Gly Asn Asn Leu Thr Ser Thr Val Gln Val Phe Ala Asp Lys Asp
        195                 200                 205
Tyr Gln Leu Pro Tyr Val Leu Gly Ser Ala Thr Glu Gly Thr Phe Pro
    210                 215                 220
```

```
Pro Phe Pro Ala Asp Ile Tyr Thr Ile Pro Gln Tyr Gly Tyr Cys Thr
225                 230                 235                 240
Leu Asn Tyr Asn Asn Glu Ala Val Asp Arg Ser Ala Phe Tyr Cys Leu
                245                 250                 255
Asp Tyr Phe Pro Ser Asp Met Leu Arg Thr Gly Asn Asn Phe Glu Phe
            260                 265                 270
Thr Tyr Thr Phe Glu Asp Val Pro Phe His Ser Met Phe Ala His Asn
        275                 280                 285
Gln Thr Leu Asp Arg Leu Met Asn Pro Leu Val Asp Gln Tyr Leu Trp
    290                 295                 300
Ala Phe Ser Ser Val Ser Gln Ala Gly Ser Ser Gly Arg Ala Leu His
305                 310                 315                 320
Tyr Ser Arg Ala Thr Lys Thr Asn Met Ala Ala Gln Tyr Arg Asn Trp
                325                 330                 335
Leu Pro Gly Pro Phe Phe Arg Asp Gln Gln Ile Phe Thr Gly Ala Ser
            340                 345                 350
Asn Ile Thr Lys Asn Asn Val Phe Ser Val Trp Glu Lys Gly Lys Gln
        355                 360                 365
Trp Glu Leu Asp Asn Arg Thr Asn Leu Met Gln Pro Gly Pro Ala Ala
    370                 375                 380
Ala Thr Thr Phe Ser Gly Glu Pro Asp Arg Gln Ala Met Gln Asn Thr
385                 390                 395                 400
Leu Ala Phe Ser Arg Thr Val Tyr Asp Gln Thr Thr Ala Thr Thr Asp
                405                 410                 415
Arg Asn Gln Ile Leu Ile Thr Asn Glu Asp Glu Ile Arg Pro Thr Asn
            420                 425                 430
Ser Val Gly Ile Asp Ala Trp Gly Ala Val Pro Thr Asn Asn Gln Ser
        435                 440                 445
Ile Val Thr Pro Gly Thr Arg Ala Ala Val Asn Asn Gln Gly Ala Leu
    450                 455                 460
Pro Gly Met Val Trp Gln Asn Arg Asp Ile Tyr Pro Thr Gly Thr His
465                 470                 475                 480
Leu Ala Lys Ile Pro Asp Thr Asp Asn His Phe His Pro Ser Pro Leu
                485                 490                 495
Ile Gly Arg Phe Gly Cys Lys His Pro Pro Pro Gln Ile Phe Ile Lys
            500                 505                 510
Asn Thr Pro Val Pro Ala Asn Pro Ser Glu Thr Phe Gln Thr Ala Lys
        515                 520                 525
Val Ala Ser Phe Ile Asn Gln Tyr Ser Thr Gly Gln Cys Thr Val Glu
    530                 535                 540
Ile Phe Trp Glu Leu Lys Lys Glu Thr Ser Lys Arg Trp Asn Pro Glu
545                 550                 555                 560
Ile Gln Phe Thr Ser Asn Phe Gly Asn Ala Ala Asp Ile Gln Phe Ala
                565                 570                 575
Val Ser Asp Thr Gly Ser Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg
            580                 585                 590
Tyr Leu Thr Lys Pro Leu
        595
```

<210> 14
<211> 1608
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 14

```
atggctgaag gaggtggcgg cccagtgggc gatgcaggcc agggtgccga tggagtgggc       60
aattcctccg gaaattggca ttgcgattcc caatggctgg aaaacggagt cgtcactcga      120
```

```
accacccgaa cctgggtctt gcccagctac aacaaccacc tgtacaaacg aatccaagga     180
cccagcggag gcgacaacaa caacaaattc tttggattca gcaccccctg ggatactttt     240
gactacaatc gattccactg ccacttttcc ccgcgagact ggcaacgact catcaacaac     300
aactggggca tccgtcccaa agcgatgcgc tttagactct ttaacatcca ggttaaagag     360
gtcacggtcc aagacttcaa caccaccatc ggcaacaacc tcaccagtac ggtccaggtc     420
tttgcggaca aggactacca actgccgtac gtcctcggat cggctaccga aggcaccttc     480
ccgccgttcc cagcggatat ctacacgatc ccgcagtacg ggtactgcac gctaaactac     540
aacaacgagg cggtggatcg ttcggccttc tactgtctgg actactttcc ctcagacatg     600
ctgcggacag gaaataactt tgagtttact tacaccttcg aggacgttcc tttccatagc     660
atgtttgccc acaaccagac gctagaccgg ctgatgaatc ccctcgtgga tcagtacctc     720
tgggctttca gctccgtcag ccaagcaggc tcatctggac gagctcttca ttactcgcgg     780
gcgactaaaa ccaacatggc ggctcaatat aggaactggt tacctgggcc tttcttccgt     840
gatcagcaaa tctttacggg cgctagcaac atcactaaaa ataacgtctt tagcgtttgg     900
gaaaaaggca agcaatggga actcgacaat cggaccaacc taatgcagcc cggtcctgcg     960
gcagcgacca cctttagcgg agaacctgac cgtcaagcca tgcaaaacac gctggctttt    1020
agcaggaccg tctacgatca aacgaccgcc acgaccgatc gtaaccagat actcatcacc    1080
aacgaagacg aaatcagacc caccaactcg gtcggtatcg acgcgtgggg agcagttccc    1140
accaacaacc agtcgatcgt gacccccggc actcgcgcgg ccgtcaacaa tcaaggggcg    1200
cttcccggga tggtgtggca aaacagagac atttacccta cagggaccca tttggccaaa    1260
attcccgaca ctgacaatca cttccatccg tccccgctta ttgggcggtt tggctgcaag    1320
catccccctc cccagatttt cattaaaaac acacccgtcc ctgccaaccc ttcggaaacg    1380
ttccagacgg ccaaagtggc ctccttcatc aaccagtact cgaccggaca gtgcaccgtc    1440
gaaatctttt gggaactcaa gaaggaaacc tccaagcgct ggaaccccga aatccagttc    1500
acctccaact ttggcaacgc ggccgacatc cagtttgccg tctccgacac gggatcctat    1560
tccgaacctc gtcccatcgg tacccgttac cttaccaaac ctctgtaa                 1608
```

<210> 15
<211> 535
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 15

```
Met Ala Glu Gly Gly Gly Gly Pro Val Gly Asp Ala Gly Gln Gly Ala
1               5                   10                  15
Asp Gly Val Gly Asn Ser Ser Gly Asn Trp His Cys Asp Ser Gln Trp
            20                  25                  30
Leu Glu Asn Gly Val Val Thr Arg Thr Thr Arg Thr Trp Val Leu Pro
        35                  40                  45
Ser Tyr Asn Asn His Leu Tyr Lys Arg Ile Gln Gly Pro Ser Gly Gly
    50                  55                  60
Asp Asn Asn Asn Lys Phe Phe Gly Phe Ser Thr Pro Trp Gly Tyr Phe
65                  70                  75                  80
Asp Tyr Asn Arg Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg
                85                  90                  95
Leu Ile Asn Asn Asn Trp Gly Ile Arg Pro Lys Ala Met Arg Phe Arg
            100                 105                 110
Leu Phe Asn Ile Gln Val Lys Glu Val Thr Val Gln Asp Phe Asn Thr
        115                 120                 125
Thr Ile Gly Asn Asn Leu Thr Ser Thr Val Gln Val Phe Ala Asp Lys
    130                 135                 140
Asp Tyr Gln Leu Pro Tyr Val Leu Gly Ser Ala Thr Glu Gly Thr Phe
145                 150                 155                 160
Pro Pro Phe Pro Ala Asp Ile Tyr Thr Ile Pro Gln Tyr Gly Tyr Cys
                165                 170                 175
Thr Leu Asn Tyr Asn Asn Glu Ala Val Asp Arg Ser Ala Phe Tyr Cys
            180                 185                 190
```

```
Leu Asp Tyr Phe Pro Ser Asp Met Leu Arg Thr Gly Asn Asn Phe Glu
        195                 200                 205
Phe Thr Tyr Thr Phe Glu Asp Val Pro Phe His Ser Met Phe Ala His
    210                 215                 220
Asn Gln Thr Leu Asp Arg Leu Met Asn Pro Leu Val Asp Gln Tyr Leu
225                 230                 235                 240
Trp Ala Phe Ser Ser Val Ser Gln Ala Gly Ser Ser Gly Arg Ala Leu
                245                 250                 255
His Tyr Ser Arg Ala Thr Lys Thr Asn Met Ala Ala Gln Tyr Arg Asn
            260                 265                 270
Trp Leu Pro Gly Pro Phe Phe Arg Asp Gln Gln Ile Phe Thr Gly Ala
        275                 280                 285
Ser Asn Ile Thr Lys Asn Asn Val Phe Ser Val Trp Glu Lys Gly Lys
    290                 295                 300
Gln Trp Glu Leu Asp Asn Arg Thr Asn Leu Met Gln Pro Gly Pro Ala
305                 310                 315                 320
Ala Ala Thr Thr Phe Ser Gly Glu Pro Asp Arg Gln Ala Met Gln Asn
                325                 330                 335
Thr Leu Ala Phe Ser Arg Thr Val Tyr Asp Gln Thr Thr Ala Thr Thr
            340                 345                 350
Asp Arg Asn Gln Ile Leu Ile Thr Asn Glu Asp Glu Ile Arg Pro Thr
        355                 360                 365
Asn Ser Val Gly Ile Asp Ala Trp Gly Ala Val Pro Thr Asn Asn Gln
    370                 375                 380
Ser Ile Val Thr Pro Gly Thr Arg Ala Ala Val Asn Asn Gln Gly Ala
385                 390                 395                 400
Leu Pro Gly Met Val Trp Gln Asn Arg Asp Ile Tyr Pro Thr Gly Thr
                405                 410                 415
His Leu Ala Lys Ile Pro Asp Thr Asp Asn His Phe His Pro Ser Pro
            420                 425                 430
Leu Ile Gly Arg Phe Gly Cys Lys His Pro Pro Pro Gln Ile Phe Ile
        435                 440                 445
Lys Asn Thr Pro Val Pro Ala Asn Pro Ser Glu Thr Phe Gln Thr Ala
    450                 455                 460
Lys Val Ala Ser Phe Ile Asn Gln Tyr Ser Thr Gly Gln Cys Thr Val
465                 470                 475                 480
Glu Ile Phe Trp Glu Leu Lys Lys Glu Thr Ser Lys Arg Trp Asn Pro
                485                 490                 495
Glu Ile Gln Phe Thr Ser Asn Phe Gly Asn Ala Ala Asp Ile Gln Phe
            500                 505                 510
Ala Val Ser Asp Thr Gly Ser Tyr Ser Glu Pro Arg Pro Ile Gly Thr
        515                 520                 525
Arg Tyr Leu Thr Lys Pro Leu
    530                 535
```

<210> 16
<211> 120
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 16

```
tggccagttt ccaagacagg ctcgctcgct cactcgggcc ggggccccaa aggggcccct      60
agcgaccgct tcgcggtcgc ggcccgagtg agcgagcgag cctgtcttgg aaactggcca     120
```

<210> 17
<211> 120
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 17
accggtcaaa ggttctgtcc gagcgagcga gtgagcccgg ccccggcgtt tccccgggga 60
tcgctgggga agcgccagcg ccgggctcac tcgctcgctc ggacagaacc tttgaccggt 120

<210> 18
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 18
gcactccggt gaggtaatgc cg 22

<210> 19
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 19
cggcattacc tcaccggagt gc 22

<210> 20
<211> 7
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 20
ccggtcg 7

<210> 21
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 21
cgagtgagcg agcgag 16

```
<210> 22
<211> 101
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
      synthetic construct

<400> 22
tcacgtggtc gggaatggga acgggaaatc tcgcgagaac gtaaacaaat ataagacggc      60
gccacacggc gctgcgtcat acgcgcgcgc gcaccggcga g                         101

<210> 23
<211> 252
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
      synthetic construct

<400> 23
aatttcgggg gcgcgaacaa ggtccgggcc gagtcgtata ttcccgccta cctgatcccg      60
aaacagcaac cggaagtgca gtgggcgtgg actaacgtgc ccgagtatat aaaagcgtgc     120
ttgcaccgag aactgcgtgc cagtctcgcg cgacttcact tcgaggaggc gggcgtctcg     180
caatccaagg aaaatctcgc gagaactgca gacggcgctc ccgtgatgcc gacccgcgtc     240
agcaaacgct ac                                                         252

<210> 24
<211> 196
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
      synthetic construct

<400> 24
gtaaggtgac caaacaggag gtgcgtcaat tcttcaggtg gtctcaggat cacctgaccc      60
ctgtgatccc agaattccta gtgcggaagg cggagtctcg caaaagaccc gccccttccg     120
gggaaggcta tataagcccg acaaagcggc ccgcgctcgc agagcagcag caggcgtcgg     180
agagcgcgga cccggt                                                     196
```